1981

cancer treatment reports

July/August 1981
Volume 65
Number 7-8
Pages 545-738

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Vincent T. DeVita, Jr, *Director*

Division of Cancer Treatment, Bruce A. Chabner, *Acting Director*

Scientific Information Branch, Susan Molloy Hubbard, *Chief*

Editorial Board

John S. Macdonald, *Editor-in-Chief*
Associate Editors:
Martin D. Abeloff
Joseph Aisner
Murray Brennan
Ross C. Donehower
Eli J. Glatstein
Daniel C. Ihde
Robert W. Makuch
Philip A. Pizzo
Charles A. Schiffer
Mary K. Wolpert
Paul V. Woolley
Daniel S. Zaharko

Editorial Staff

Jean Griffin Baum, *Managing Editor*
Elaine Price Beck, *Assistant Editor*
Dee Kittner LeRoy, *Assistant Editor*
Anne McCarthy, *Assistant Editor*
Marian Sharp McGrath, *Assistant Editor*
Virginia T. Ascosi, *Editorial Assistant*
Deidre A. Hoehn, *Editorial Assistant*
Florence L. Barnes

Advisory Board

Robert S. Benjamin
Isadore Cohn, Jr
Robert L. Goodman
Ken R. Harrap
Edward Henderson
E. Carmack Holmes
Randall K. Johnson
Irwin H. Krakoff
Robert B. Livingston
Charles G. Moertel
John H. Moxley, III
William L. McGuire
David A. Pistenma
Sydney E. Salmon
Vainutis K. Vaitkevicius

Editorial Policy

The Editorial Board will consider manuscripts dealing with all aspects of preclinical and clinical cancer treatment. These may include such topics as surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
3. **Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.**
4. **Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.**
   (a) Ideas relating to cancer treatment.
   (b) Preliminary research reports.
   (c) Clinical case reports.
   (d) Brief clinical trials of novel treatments.

All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $47.00 domestic, with an additional $11.75 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" (the identifying code for this journal) when requesting a subscription.

INSTRUCTIONS TO AUTHORS

Summary
Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus*: author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Abbreviations
Only standard abbreviations are acceptable. If a new or coined abbreviation is used, it must be defined when first mentioned.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Letters
Letters published in this section can be no longer than 300 words and can be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables suggested for Clinical Trials Letters include one of the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease [including subtypes or grade], prior therapy [number of previous regimens failed]); or *drug regimen* (dose and schedule, particularly for combination or complex regimens, number of cycles or doses/patient [mean, median, range]); or *toxicity* (standardized toxicity grades [ECOG or CALGB] or individualized grades devised and defined in the text [should demonstrate that an appropriate, biologically active dose was administered]).

Submission of Manuscripts
Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20014 (301-496-6975). We invite all authors to submit with their manuscripts a short list of possible reviewers. The editorial board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about three weeks after publication of the article.

Contents

Cancer Treatment Reports
Volume 65, Number 7–8
July/August 1981

Page

ARTICLES

Clinical and Preclinical Pharmacology

Pharmacokinetics of Leucovorin Rescue Using a New Methotrexate-Independent Biochemical Assay for Leucovorin and N^5-Methyltetrahydrofolate 545
Ernest Hamel, Gloria Johnson, and Daniel Glaubiger
National Cancer Institute, Bethesda, Maryland

Differential Pulse Polarographic Determination of BCNU Pharmacokinetics in Patients With Lung Cancer 555
Raimondo Russo, Ivan Bartošek, Elena Piazza, Anna Maria Santi, Arnaldo Libretti, and Silvio Garattini
Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy

Adrenal Response to Serial Cosyntropin Stimulation After Repeated High-Dose Prednisone Administration in Patients With Lymphoma 563
Kenneth Zamkoff, Jeffrey Kirshner, Dianne Cass, and Myron Miller
Veterans Administration Medical Center and the Upstate Medical Center, Syracuse, New York

Enhanced Cytostatic Effectiveness of Aniline Mustard Against 7,12-Dimethylbenz[a]anthracene-Induced Rat Mammary Tumors During Regression in Response to Ovariectomy 567
Catherine Benckhuysen, Hennie G. J. Ter Hart, and Pim J. Van Dijk
Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Institute, Amsterdam

Efficacy of Piperazinedione Prior to Bone Marrow Transplantation: Studies in a Rat Model for Human Acute Myelocytic Leukemia 575
Anton Hagenbeek and Anton C. M. Martens
Radiobiological Institute TNO, Rijswijk, The Netherlands

Experimental Studies on the Radiation-Modifying Effect of Bleomycin in Malignant and Normal Mouse Tissue In Vivo 583
Jeanne Molin, Poul Erik Søgaard, and Jens Overgaard
The Institute of Cancer Research, Radiumstationen, Aarhus C, Denmark

Synergistic Antileukemic Activity of Combinations of Two Nitrosoureas 591
Glynn P. Wheeler, Frank M. Schabel, Jr, and Mary W. Trader
Southern Research Institute, Birmingham, Alabama

Page

Clinical Studies

Effect of Chemotherapy for the Dysmyelopoietic Syndrome 601
James O. Armitage, Fred R. Dick, Samuel W. Needleman, and C. Patrick Burns
University of Iowa College of Medicine, Iowa City

Chromosome Analyses of Nurses Handling Cytostatic Agents 607
H. Waksvik, O. Klepp, and A. Brøgger
Norsk Hydro's Institute for Cancer Research, Oslo, Norway

Treatment of Recurrent Lymphomas With Vincristine, BCNU, Doxorubicin, and Prednisone (VBAP): A Southwest Oncology Group Study 611
J. K. Weick, S. E. Jones, A. Haut, R. Fisher, and D. Dixon
Southwest Oncology Group

Iv Hyperalimentation and Chemotherapy for Stage III Testicular Cancer: A Randomized Study 615
Melvin L. Samuels, Debra E. Selig, Sheryl Ogden, Cynthia Grant, and Barry Brown
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Radionuclide Ejection Fraction in Doxorubicin Cardiotoxicity 629
Graeme W. Morgan, Brenda M. McIlveen, Albert Freedman, and I. Provan C. Murray
The Prince of Wales Hospital, Randwick, Sydney, NSW Australia

Intensive Induction Chemotherapy in 54 Patients With Small Cell Carcinoma of the Lung 639
Martin D. Abeloff, David S. Ettinger, Stanley E. Order, Nagi Khouri, E. David Mellits, Neal T. Dorschel, and Rosemary Baumgardner
The Johns Hopkins Hospital, Baltimore, Maryland

Phase I–II Trials

Phase I Trial of Chlorozotocin: Attempted Amelioration of Myelotoxicity by Glucose Administration 647
L. Panasci, R. Comis, S. Ginsberg, L. Kohn, A. Fitzpatrick, M. Rubert, and T. Scalzo
Upstate Medical Center and Veterans Administration Medical Center, Syracuse, New York

Iv Dichloromethylene Diphosphonate in Cancer-Associated Hypercalcemia: A Phase I–II Evaluation 651
A. I. Cohen, J. Koeller, T. E. Davis, and D. L. Citrin
University of Wisconsin, Madison

Phase II Study of AMSA in Lung Cancer 655
Michael K. Samson, Roberto J. Fraille, Laurence H. Baker, Glenn Cummings, and Robert W. Talley
Wayne State University School of Medicine, Detroit, Michigan

Page

Phase II Study of Vindesine in Patients With Metastatic Breast Cancer 659
Melody A. Cobleigh, Stephen D. Williams, and Lawrence H. Einhorn
Indiana University School of Medicine, Indianapolis

BRIEF REPORTS AND PRELIMINARY COMMUNICATIONS

Kinetics of Cisplatin in an Anuric Patient Undergoing Hemofiltration Dialysis 665
Alain Gouyette, Roland Lemoine, Jean-Pierre Adhemar, Dieter Kleinknecht, Ngyen-Khoa Man, Jean-Pierre Droz, and Jean-Pierre Macquet
Institut Gustave-Roussy, Villejuif, France

Pharmacokinetics of Hexamethylmelamine and Pentamethylmelamine in Mice 669
M. Broggini, T. Colombo, M. D'Incalci, M. G. Donelli, A. Gescher, and S. Garattini
Istituto di Richerche Farmacologiche "Mario Negri," Milan, Italy

Antitumor Activity of Tuftsin on Murine Melanoma In Vivo 673
R. Dirk Noyes, George F. Babcock, and Kenji Nishioka
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Venous Thrombosis During Multimodal Treatment of Primary Breast Carcinoma 677
Raymond B. Weiss, Douglass C. Tormey, James F. Holland, and Vivian E. Weinberg
Cancer and Leukemia Group B

Adjuvant Chemotherapy for Medulloblastoma and Ependymoma Using Iv Vincristine, Intrathecal Methotrexate, and Intrathecal Hydrocortisone: A Southwest Oncology Group Study 681
Jan van Eys, Timothy Chen, Terrence Moore, William Cheek, Charles Sexauer, and Kenneth Starling
Southwest Oncology Group

Laparotomy in the Re-evaluation of Patients With Advanced Hodgkin's Disease 685
Nina C. Kostraba, Bruce A. Peterson, B. J. Kennedy, Theodor B. Grage, and Clara D. Bloomfield
University of Minnesota Health Sciences Center and the Masonic Cancer Center, Minneapolis

Treatment of Renal Carcinoma: A Phase III Randomized Trial of Oral Medroxyprogesterone (Provera), Hydroxyurea, and Nafoxidine 689
Leo L. Stolbach, Colin B. Begg, Thomas Hall, and John Horton
Eastern Cooperative Oncology Group

Page

Ifosfamide: A Weekly Dose Fractionated Schedule in Bronchogenic Carcinoma 693
L. R. Morgan, L. E. Posey, J. Rainey, J. Bickers, D. Ryan, R. Vial, and E. W. Hull
Louisiana State University Medical Center, New Orleans

Combined Radiotherapy and Bleomycin for Advanced Head and Neck Cancers: Results of a Phase I Pilot Study 697
Ivan J. Silverberg, Theodore L. Phillips, Karen K. Fu, and Paul Y. M. Chan
University of California, San Francisco

Neocarzinostatin: Report of a Phase II Clinical Trial 699
Eugene M. McKelvey, William Murphy, Axel Zander, and Gerald P. Bodey
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Oral VP-16-213 in Transitional Cell Carcinoma of the Bladder: A Phase II Study 703
Jytte Panduro, Mogens Hansen, and Heine Høi Hansen
Radiumstationen, Finsen Institute, Copenhagen, Denmark

Phase II Evaluation of Chlorozotocin in Patients With Malignant Melanoma 705
Alan N. Houghton, Fernando J. Camacho, Richard J. Gralla, and Robert Wittes
Memorial Sloan-Kettering Cancer Center, New York, New York

Phase I Study With Cell Wall Skeleton of *Nocardia rubra* 707
Yuichi Yamamura, Takeshi Ogura, Fumio Hirao, Kosei Yasumoto, Kenji Sawamura, Shoji Hattori, Yoshihiro Hayata, Susumu Kishimoto, Kazumasa Yamada, Hisanobu Niitani, and Toru Masaoka
Osaka University Medical School, Japan

Phase II Study of Vinblastine-CCNU, Triazinate, and Dactinomycin in Advanced Renal Cell Cancer 711
Richard G. Hahn, Colin B. Begg, and Thomas Davis
Eastern Cooperative Oncology Group

Evaluation of VP-16-213, Cyclophosphamide, Doxorubicin, and Cisplatin (V-CAP) in Advanced Large Cell Lung Cancer 715
Robert T. Eagan, Stephen Frytak, William C. Nichols, James N. Ingle, Edward T. Creagan, Larry K. Kvols, and Douglas T. Coles
Mayo Clinic, Rochester, Minnesota

MEETING REPORT

Working Conference on Neuroblastoma Treatment Trials 719
Richard S. Ungerleider
National Cancer Institute, Bethesda, Maryland

Page

LETTERS

Anaphylactic Reaction to Methotrexate Used in High Doses as an Adjuvant Treatment of Osteogenic Sarcoma 725
P. Klimo and E. Ibrahim
Cancer Control Agency of British Columbia, Vancouver, Canada

Baker's Antifol in Children With Therapy-Resistant Solid Tumors: A Southwest Oncology Group Study 725
R. Nitschke, S. K. Morgan, and V. J. Land
Southwest Oncology Group

Pulmonary Toxicity Studies of Bleomycin Tested Before and After Its Expiration Date 727
Abraham Schlein, John E. Schurig, Charles D. Baca, William T. Bradner, and Stanley T. Crooke
Bristol Laboratories, Syracuse, New York

Effect of Tamoxifen in Premenopausal Japanese Women With Advanced Breast Cancer 728
Tomio Wada, Hiroki Koyama, and Toshio Terasawa
The Center for Adult Diseases, Osaka, Japan

CLINICAL TRIALS LETTERS

Double Alkylator Therapy (Thiotepa Plus Chlorambucil) for Previously Treated Advanced Ovarian Cancer 731
Robert F. Ozols, Diane M. Howser, and Robert C. Young
National Cancer Institute, Bethesda, Maryland

Phase II Study of Cyclophosphamide and Hexamethylmelamine in Non-Oat Cell Bronchogenic Carcinoma 731
Maria Brambilla Pisoni, Arnaldo Libretti, Elena Piazza, Giuseppe Vago, Roberto Scapaticci, Celso Bianchi, Maurizio Tomirotti, Alberto Scanni, Maria Pia Calzavara, Lucia Cassani, Giorgio Scarpazza, Silvia Marsoni, and Luciano Morasca
North Milan Cooperative Group

Sequential Combination Chemotherapy for Disseminated Melanoma: A Southwest Oncology Group Study 732
John Costanzi, Carol Fabian, Henry Wilson, and Dennis Dixon
Southwest Oncology Group

Phase II Study of Chlorozotocin in Non-Small Cell Carcinoma of the Lung 734
Cornelius J. Cornell, Jr, Daniel Hoth, and Thomas F. Pajak
Cancer and Leukemia Group B

Phase II Trial of 5-FU Plus Melphalan in Advanced Colorectal Carcinoma 735
I. R. Gough, C. M. Furnival, G. S. J. Thynne, and W. Burnett
University of Queensland, Royal Brisbane Hospital, Brisbane, Australia

Page

ANNOUNCEMENTS 737

Oral Complications of Cancer Chemotherapy
Eighth Annual Conference on Medical Oncology
San Antonio Fourth Annual Breast Cancer Symposium
Current Concepts in Cancer Management: Successful Treatment and Its Consequences
Revisions for the Directory of Training Programs in Internal Medicine: Residency and Subspecialty Fellowships

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Pharmacokinetics of Leucovorin Rescue Using a New Methotrexate-Independent Biochemical Assay for Leucovorin and N^5-Methyltetrahydrofolate[1]

Ernest Hamel, Gloria Johnson, and Daniel Glaubiger[2,3,*]

SUMMARY

A rapid biochemical assay for leucovorin (LV) and N^5-methyltetrahydrofolate (mTHFA) has been developed, using a cell-free extract of *Escherichia coli*. The reaction used was the formylation of [^{14}C]methionyl-tRNA $^{fMet}_{E.\ coli}$, in which product formation is dependent on added folate derivatives. The actual formyl donor, N^{10}-formyltetrahydrofolate, is generated from LV in the presence of ATP or from mTHFA in the presence of NAD^+ or $NADP^+$. The assay is sensitive to approximately 5×10^{-8} M *l*-LV and 5×10^{-7} M *l*-mTHFA in serum. There is no cross-reactivity between LV and mTHFA. The presence of methotrexate (MTX) has no effect on assay results. This assay has been used to determine LV and mTHFA levels in patients receiving LV rescue after high-dose MTX infusions. Patients received 96 mg/m^2 of LV as a single iv dose at the conclusion of the MTX infusion. Serum levels of LV and mTHFA were followed for the next 6 hrs. The initial (5-min) level of LV was about 10^{-5} M, and its concentration in the serum decreased rapidly. The α-phase half-life of LV was about 15 mins, but LV was readily detectable by the assay for 3–4 hrs. There was rapid apparent conversion to mTHFA, as this compound was also detectable at the initial time point. The level of mTHFA increased for at least 60 mins, being equimolar with LV by 30 mins, and then decreased slowly with an apparent half-life of 2–3 hrs.

[Cancer Treat Rep 65:545–553, 1981]

In recent years, high-dose methotrexate (MTX) has been used with increasing frequency for the treatment of many human malignancies (1). Such therapy has required the concomitant use of a "rescue" agent to minimize the toxic effects of MTX. The rescue agent generally used has been leucovorin (LV). While the pharmacokinetics of MTX are readily followed (2), there has been no generally available and rapid method to measure LV. The pharmacokinetics of the rescue phase of high-dose MTX therapy have therefore not been explored in detail.

In this report we describe a simple biochemical assay for LV, based on the formylation of bacterial initiator methionyl-tRNA (Met-tRNA) in a cell-free extract derived from *Escherichia coli*. This assay is not only MTX-independent but also able to distinguish between LV and N^5-methyltetrahydrofolate (mTHFA), the major folate derivative in human serum (3). The distinction between LV and mTHFA is based on differing cofactor requirements for their conversion to the active donor species. ATP is required for the conversion of LV, and $NADP^+$ is required for the conversion of mTHFA. We have used this assay in a preliminary study of the phar-

[1]Received May 30, 1980; revised Nov 24, 1980; accepted Dec 31, 1980.

[2]Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]We thank Dr. B. A. Chabner for a critical review of the manuscript.

*Reprint requests to: Daniel Glaubiger, MD, Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 3B04, National Institutes of Health, Bethesda, MD 20205.

macokinetics of LV disappearance and mTHFA formation after an iv dose of LV in several patients.

MATERIALS AND METHODS

Materials

The following substances were obtained from various sources: frozen *E. coli* B (half-log phase) from Grain Processing Corp; [^{14}C]methionine (specific activity, 285 curies/mol) from Amersham; *E. coli* tRNA from Schwartz-Mann; LV, mTHFA, ATP, and $NADP^+$ from Sigma; DNase from Worthington; and MTX from Ben Venue Laboratories (supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD).

Preparation of Supernatant Enzymes (S100)

Twenty grams of *E. coli* was ground with 40 g of washed alumina, and the cell paste was extracted with 40 ml of a buffer containing 10 mM Tris-HCl (pH 7.4), 40 mM KCl, 10 mM $MgCl_2$, and 2 mM 2-mercaptoethanol (buffer A). After hydrolysis of DNA with 2 μg of DNase for 10 mins, cell debris and alumina were removed by centrifugation at 30,000 × *g* for 15 mins, and ribosomes were removed by centrifugation at 100,000 × *g* for 3 hrs. The supernatant was passed through a Sephadex G-25 column (5 × 30-cm) and then precipitated with 70% $(NH_4)_2SO_4$. The column was equilibrated and the sample was eluted with buffer A. The precipitate was dissolved in 40 ml of a buffer containing 10 mM Tris-HCl (pH 7.4) and 2 mM 2-mercaptoethanol, was dialyzed against the same buffer for 16 hrs with one buffer change, and was stored frozen in small aliquots in a liquid nitrogen freezer. All operations were performed at 0–5°C.

Preparation of [^{14}C]Met-tRNA

A 100-ml reaction mixture was prepared containing 100 mM Tris-HCl (pH 7.7), 15 mM $MgCl_2$, 15 mM 2-mercaptoethanol, 7.5 mM ATP, 200 microcuries of [^{14}C]methionine, 400 mg of *E. coli* tRNA, and 10 ml of S100. This mixture was incubated for 40 mins at 37°C, chilled in ice, and extracted with an equal volume of water-saturated phenol. The phases were separated, and the phenol phase was re-extracted with 50 ml of water. The two aqueous phases were pooled, and the [^{14}C]Met-tRNA was precipitated at –20°C by the addition of 7.5 ml of saturated potassium acetate (pH 5.0) and 315 ml of ethanol. The [^{14}C]Met-tRNA was collected by centrifugation, dissolved in 0.1 mM $MgCl_2$, passed through a Sephadex G-25 column, reprecipitated as before with potassium acetate and ethanol, recentrifuged, dissolved in and dialyzed against 0.1 mM $MgCl_2$, and stored frozen in aliquots at a final concentration of 240 A_{260} units/ml.

Formylation of [^{14}C]Met-tRNA

The 50-μl reaction mixtures contained 10 μl of S100, 20 mM 2-mercaptoethanol, 50 pmols of [^{14}C]Met-tRNA (about 10,000 cpm), 100 mM Tris-HCl (pH 7.7), and as indicated in the individual experiments, $CaCl_2$, $MgCl_2$, mTHFA, LV, ATP, $NADP^+$, human serum, and MTX. The reaction mixtures were incubated for 10 mins at 37°C, the reaction being approximately 60%–70% complete with saturating LV under these conditions. The time course of the reaction was hyperbolic, presumably because Met-tRNA was present in limiting amounts.

The reactions were stopped with 0.5 ml of 0.1 M KOH. After degradation of the tRNA by a 30-min incubation at 37°C, the reaction mixtures were acidified with 0.1 ml of concentrated HCl (12 N). After addition of 1.5 ml of ethyl acetate, the reaction samples were vigorously vortexed for 5 secs, and the phases were separated by centrifugation at 3000 rpm for 2 mins. The radioactivity in 1 ml of the ethyl acetate phase was measured in a liquid scintillation counter. Unreacted methionine remained in the aqueous phase while *N*-formylmethionine was quantitatively extracted into the ethyl acetate phase [(4), unpublished observations]. Assays were always performed in duplicate. Data in all tables are corrected for the amount of [^{14}C]methionine extracted into the ethyl acetate phase in the absence of S100. This value ranged from 11.7 to 14.2 pmols/ml (average, 12.7). The data in the figures are not corrected.

Patient Studies

Pharmacokinetics were studied in four patients: two with advanced metastatic osteosarcoma and two with abdominal lymphomas. All patients were receiving frequent 42-hr high-dose MTX infusions as part of treatment protocols. Patients received 600 mg/m^2 of MTX iv during the first hour and then 120 mg/m^2/hr iv for the next 41 hrs. At 42 hrs a serum sample was drawn and used as a baseline specimen. The MTX infusion was stopped, and 96 mg/m^2 of LV was given iv over 5–10 mins. Serum specimens were then drawn at the indicated times.

RESULTS

Folate Interconversions and Formylation of Met-tRNA$_{E.\ coli}^{fMet}$

Figure 1 summarizes the metabolic conversions of LV and mTHFA to N^{10}-formyltetrahydrofolate (5), the actual formyl donor in the formylation of bacterial initiator Met-tRNA (Met-tRNA$_{E.\ coli}^{fMet}$) (6). mTHFA is first converted to N^5,N^{10}-methylenetetrahydrofolate with a requirement of NAD^+ as cofactor. This compound is in turn converted to N^5,N^{10}-methenyltetrahydrofolate with $NADP^+$ required as cofactor. LV is also converted to N^5,N^{10}-methenyltetrahydrofolate, but this is an irreversible reaction and has a cofactor requirement for ATP. N^5,N^{10}-Methenyltetrahydrofolate is enzymatically converted to the formyl donor, N^{10}-formyltetrahydrofolate. The interaction of Met-tRNA$_{E.\ coli}^{fMet}$ and N^{10}-formyltetrahydrofolate is a simple formyl exchange reaction with formation of N-formyl-Met-tRNA$_{E.\ coli}^{fMet}$ and tetrahydrofolate (6).

The known effectiveness of LV as a formyl donor for Met-tRNA$_{E.\ coli}^{fMet}$ (7) and the existence of a simple and highly selective means of separating methionine and N-formylmethionine (4) led us to explore the possibility of using this reaction sequence as a rapid assay for both LV and mTHFA.

Table 1 summarizes the cofactor requirements of the formylation reaction when [^{14}C]Met-tRNA is used as substrate and *E. coli* S100 is used as the enzyme source. Little activity is seen in the absence of any cofactor. Minimal stimulation is seen when a single cofactor, whether LV, mTHFA, ATP, or $NADP^+$, is added. Similarly, little activity is seen with either the combination of mTHFA and ATP or that of LV and $NADP^+$. However, a brisk reaction occurs with LV and ATP, and a moderate reaction occurs with mTHFA and $NADP^+$, as predicted by the metabolic pathways shown in figure 1. We found that either $NADP^+$ or NAD^+ was effective as a cofactor with mTHFA, but we were unable to demonstrate any synergism between the two. The data in table 1 clearly demonstrate that this assay method distinguishes between LV and mTHFA on the basis of their distinct cofactor requirements in the formylation of Met-tRNA.

Table 1 also shows the effect of 10% normal human serum on the formylation of Met-tRNA. The reaction dependent on LV and ATP is significantly inhibited by serum, while that dependent on mTHFA and $NADP^+$ is not. In some experiments, including that shown in table 1, serum appeared to have a stimulatory effect on the mTHFA-dependent reaction. Serum alone, without added folate, does not support the reaction.

Figure 2 presents a concentration study with *dl*-LV, both with and without 10% serum. Serum, although inhibitory at all concentrations, had little effect on the overall shape of the concentration curve. The limits of the sensitivity of the assay appear to be approximately 1–2 $\times$ 10^{-8} M. Since this represents the concentration of *dl*-LV, the assay is presumably sensitive to approximately 5 $\times$ 10^{-9} M *l*-LV, the biologically active isomer (8). The practical limit of sensitivity in serum is therefore approximately 5 $\times$ 10^{-8} M when 10% serum is used. It should be noted that in the presence of $CaCl_2$ (fig 1), as opposed to $MgCl_2$ (table 1), the assay was somewhat more sensitive. Serum was also less inhibitory when $CaCl_2$ was used instead of $MgCl_2$.

Figure 3 presents a concentration study with *dl*-mTHFA, with and without 10% serum. There is essentially no difference between the two curves. The limit of sensitivity of the assay is approximately 1–2 $\times$ 10^{-7} M *dl*-mTHFA. Again, since presumably only *l*-mTHFA is active, this represents a true sensitivity for the assay of approximately 10^{-7} M. The sensitivity of the assay in serum is therefore about 10^{-6} M *l*-mTHFA when 10% serum is used and 5 $\times$ 10^{-7} M with 20% serum.

Table 2 demonstrates that both ATP-dependent and $NADP^+$-dependent formylation of Met-tRNA occurs when serum obtained after LV infusion is added to the assay system. Shortly after an iv bolus of LV, ATP-dependent activity, presumably representing LV, and $NADP^+$-dependent activity, presumably representing mTHFA, are observed. The ATP-dependent activity decreases rapidly after the bolus, while the $NADP^+$-dependent activity initially increases and then decreases relatively slowly, as discussed below.

Since iv, im, or orally administered LV is partially converted to mTHFA [table 2, (9,10)], it is important to demonstrate that the LV-dependent reaction is not inhibited by mTHFA and that the mTHFA-dependent reaction is not inhibited by LV. The data in table 3 demonstrate that this is the case in both the presence and absence of serum. The LV-dependent reaction is unaffected by a tenfold excess of mTHFA; the mTHFA-dependent reaction is unaffected by a tenfold excess of LV.

Effect of MTX

To be clinically useful, an assay for LV and mTHFA must be essentially unaffected by the presence of MTX. Table 4 demonstrates that this is true for the formylation of Met-tRNA. Increasing amounts of MTX had little effect on either the LV- or mTHFA-dependent reaction whether or not serum was pres-

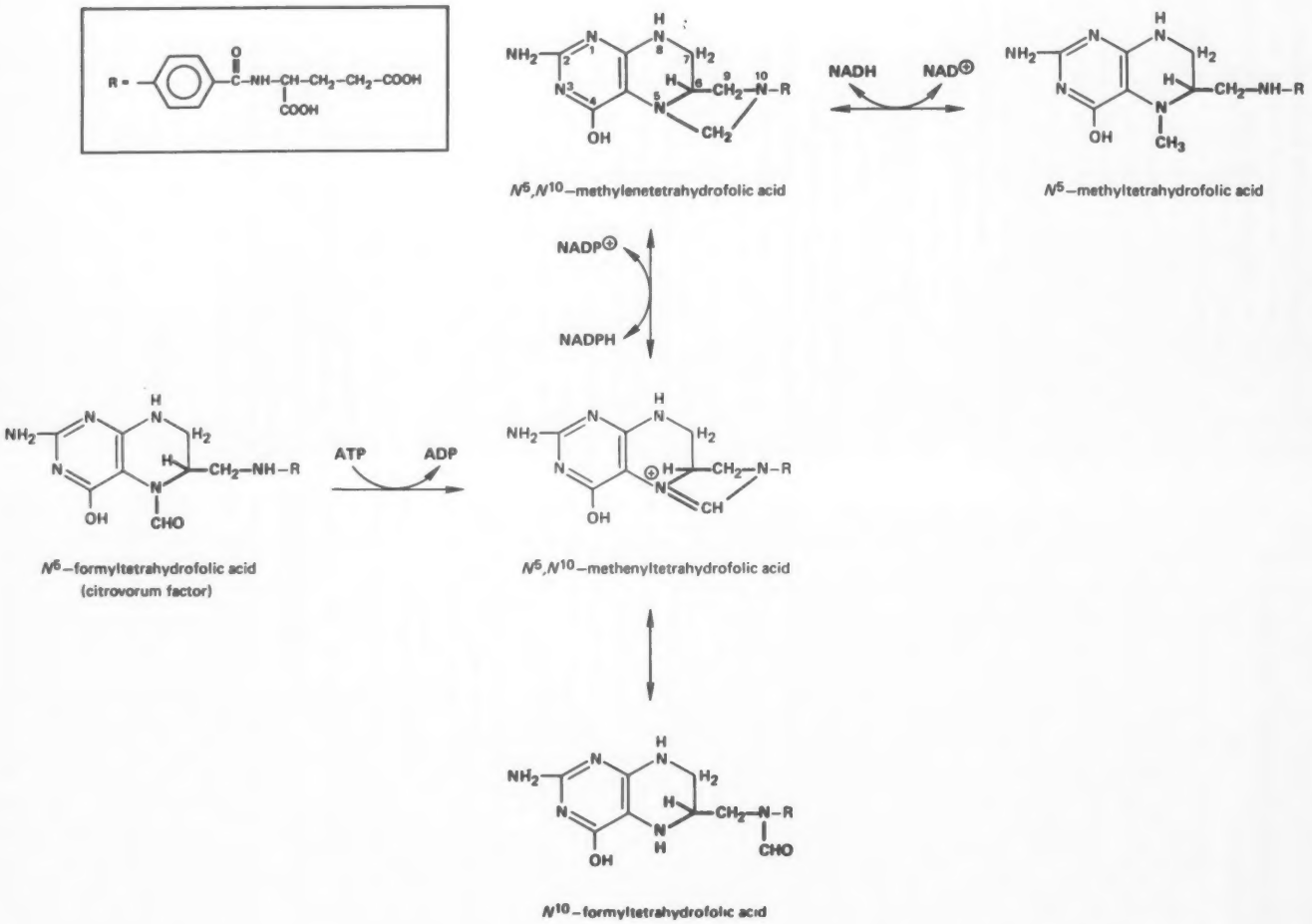

FIGURE 1.—Metabolic interconversions of folate derivatives. LV requires ATP in order to be converted to N^{10}-formyltetrahydrofolate, while mTHFA requires NAD^+ and $NADP^+$.

TABLE 1.—ATP or $NADP^+$ requirement and the effect of serum on Met-$tRNA_{E.\ coli}^{Met}$ formylation*

Cofactors added	Cofactors added in experiment 1				Cofactors added in experiment 2			
	None		LV		None		mTHFA	
	−Serum	+Serum	−Serum	+Serum	−Serum	+Serum	−Serum	+Serum
None	2.0	3.9	5.3	2.9	0.4	0.1	4.6	2.8
ATP	10.1	5.7	432.0	228.0	—	—	10.7	2.2
$NADP^+$	7.6	11.7	11.5	12.0	—	—	47.4	66.4

*Each 50-μl reaction mixture contained the components described in the text, 10 mM $MgCl_2$, and the following (when indicated above): 5 μl of serum, 6 mM ATP, 10 mM $NADP^+$, 1 μM LV, and 1 μM mTHFA. Samples were incubated and processed as described in the text. Data are expressed as pmols methionine/ml of reaction mixture extracted into the ethyl acetate phase and are corrected for the methionine extracted in the absence of S100.

ent, except that very high (10^{-3} M) MTX levels mildly inhibited the mTHFA-dependent reaction.

Pharmacokinetics of LV and mTHFA After an Iv Bolus of LV

In a preliminary pharmacokinetic study, four patients receiving high-dose MTX for treatment of advanced malignancies were studied in detail during the initial phase of the rescue period (fig 4). Serum drawn just prior to the iv bolus of LV was used to generate standard LV and mTHFA curves similar to those displayed in figures 2 and 3. This baseline serum had no significant activity in either reaction in the absence of added LV or mTHFA. Blood samples were taken at the indicated times after rapid infusion of 96 mg/m^2 of LV. ATP-dependent activity in these specimens was taken to represent LV; $NADP^+$-dependent activity was taken to represent mTHFA. Serum concentrations of LV and mTHFA at the indicated times were derived from the standard curves obtained in the presence of the baseline serum. The values given in figure 4 are those calculated for the *l*-isomers of LV and mTHFA. The serum specimens obtained after LV infusion had little activity in the absence of added ATP or $NADP^+$ (table 2). LV levels were followed in all four pa-

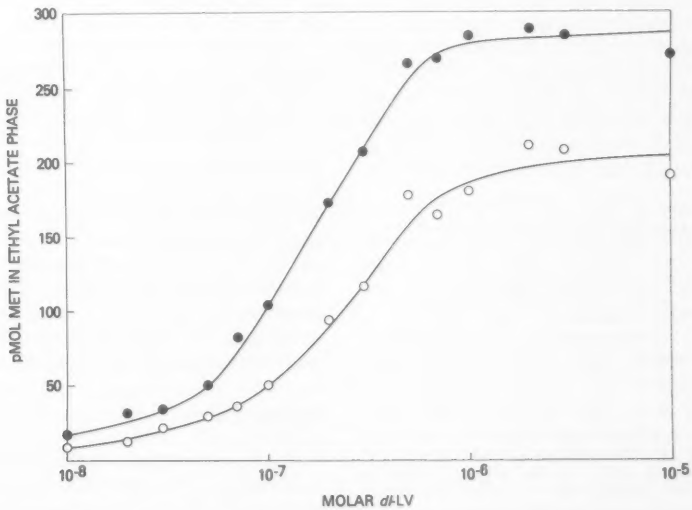

FIGURE 2.—Effect of LV concentration on the formylation of [^{14}C]Met-tRNA. Each 50-μl reaction mixture contained the indicated concentration of LV, 5 μl of serum (○) or no serum (●), the components described in the text, 5 mM $CaCl_2$, and 6 mM ATP. Samples were incubated and processed as described in the text. Data are expressed as pmols/ml of reaction mixture.

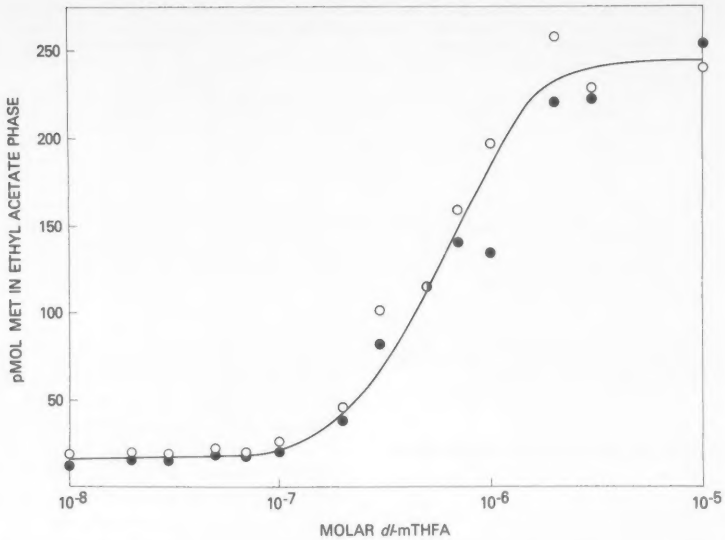

FIGURE 3.—Effect of mTHFA concentration on the formylation of [^{14}C]Met-tRNA. Each 50-μl reaction mixture contained the indicated concentrations of mTHFA, 10 μl of serum (○) or no serum (●), the components described in the text, 10 mM $MgCl_2$, and 10 mM $NADP^+$. Samples were incubated and processed as described in the text. Data are expressed as pmols/ml of reaction mixture.

tients; mTHFA levels were followed only in the two patients with lymphoma.

MTX levels decreased relatively slowly with a half-life of approximately 2 hrs. In contrast, LV levels (ATP-dependent activity) decreased rapidly with a half-life of about 15 mins, and by 3–4 hrs LV was not detectable in the patients' serum. A significantly lower level of mTHFA ($NADP^+$-dependent activity) than LV was found initially. mTHFA levels increased rapidly and by 30 mins equaled the LV level. Unlike LV, however, mTHFA persisted at a relatively high concentration over the time studied, decreasing slowly with a half-life at least as long as that of MTX.

TABLE 2.—Requirement for $NAPD^+$ or ATP for formylation of Met-$tRNA_{E.\ coli}^{Met}$ in the presence of serum obtained after LV infusion*

Cofactor added	Serum obtained—	
	Before LV infusion	After LV infusion
None	2.5	16.2
ATP	5.0	270.3
$NADP^+$	8.2	69.9

*Each 50-μl reaction mixture contained the components described in the text, 10 mM $MgCl_2$, and the following (when indicated above): 6 mM ATP, 10 mM $NADP^+$, 5 μl of serum obtained either before LV infusion or 15 mins after LV infusion as described in the text. Samples were incubated and processed as described in the text. Data are expressed as pmols methionine/ml of reaction mixture extracted into the ethyl acetate phase and are corrected for the methionine extracted in the absence of S100.

A number of investigators have reported that folate derivatives in serum samples appear to deteriorate but are at least partially preserved by ascorbic acid (11–13). We found that only $NADP^+$-dependent activity slowly deteriorated with storage and that this deterioration was only partially prevented by ascorbic acid. The studies presented in figure 4 were therefore performed on the days the sera were drawn. Ascorbic acid did not affect the assays themselves, either with patient samples or with the standard solutions. The LV standards in water were stable when stored frozen. The mTHFA standards, however, deteriorated with storage and were discarded after 2 weeks.

DISCUSSION

We have developed a simple cell-free biochemical assay for LV and mTHFA, two clinically important folate derivatives. The assay is unaffected by MTX and is sensitive to about 5×10^{-8} M *l*-LV and 5×10^{-7} M *l*-mTHFA in serum. Several other MTX-independent radioimmune and microbiologic assays for LV and mTHFA have been described (10,13–

TABLE 3.—Lack of effect of mTHFA and LV on the reactions dependent on the alternate folate cofactor*

Experiment No.	Cofactors added	−Serum	+Serum
1	None	3.1	2.4
	1 μM LV + ATP	321.7	208.7
	1 μM LV + ATP + 10 μM mTHFA	333.7	192.5
2	None	2.7	2.2
	1 μM mTHFA + $NADP^+$	122.1	119.5
	1 μM mTHFA + $NADP^+$ + 10 μM LV	121.5	116.1

*Each 50-μl reaction mixture contained the components described in the text, 5 mM $CaCl_2$ in experiment 1, 10 mM $MgCl_2$ in experiment 2, and the following (when indicated above): 5 μl of serum, 6 mM ATP, 10 mM $NADP^+$, and LV and mTHFA at the indicated concentrations. Samples were incubated and processed as described in the text. Data are expressed as pmols methionine/ml of reaction mixture extracted into the ethyl acetate phase and are corrected for the methionine extracted in the absence of S100.

TABLE 4.—Inability of MTX to affect Met-tRNA$^{Met}_{E.\ coli}$ formylation*

	Folate derivatives added					
	None		10^{-6} M LV		10^{-6} M mTHFA	
MTX concentration	−Serum	+Serum	−Serum	+Serum	−Serum	+Serum
None	9.5; 6.8†	5.9; 12.1	393.6	223.7	119.9	114.4
10^{-6} M	—	—	396.5	193.7	112.0	113.4
10^{-5} M	—	—	386.8	194.7	110.3	104.0
10^{-4} M	7.9; 6.5	7.3; 10.1	363.7	195.7	108.7	107.7
10^{-3} M	—	—	364.9	196.6	70.9	81.1

*Each 50-μl reaction mixture contained the components described in the text, 10 mM $MgCl_2$, as well as the following (when indicated above): MTX, LV, and mTHFA at the indicated concentrations; 5 μl of serum; 6 mM ATP in samples containing LV; and 10 mM $NADP^+$ in samples containing mTHFA. Samples were incubated and processed as described in the text. Data are expressed as pmols methionine/ml of reaction mixture extracted into the ethyl acetate phase and are corrected for the methionine extracted in the absence of S100.

†Samples contained either 6 mM ATP (1st entries) or 10 mM $NADP^+$ (2nd entries).

16). The biochemical assay described here, although less sensitive, has several advantages.

Perhaps most importantly, the entire assay can be performed rapidly. Incubation time is only 10 mins, and standard curves and patient specimens can be processed in 2–4 hrs, depending on the number of samples. Second, all components required are commercially available or easily prepared from commercially available materials. Third, LV standards, Met-tRNA, and *E. coli* S100 can all be stored frozen until needed, with only the mTHFA standards requiring recent preparation. Finally, the assay is unaffected by the presence of MTX (table 4).

The data show that baseline serum has minimal activity with either ATP or $NADP^+$ in the formylation of Met-tRNA. After infusion of LV, patients develop a great deal of ATP-dependent activity in their serum. This activity, representative of LV, disappears rapidly with a half-life of 15 mins.

Patients also develop substantial $NADP^+$-dependent activity in their serum following LV infusion. This activity increases over the first 60 mins, remains elevated over the 6-hr period studied, and has a slow disappearance curve. This sustained plateau is somewhat surprising but may be the result of uptake of the infused LV by the tissues, with subsequent release of the metabolic product mTHFA. Other investigators have, in fact, shown that little folate is excreted after an iv dose of LV (9,17) and that mTHFA is conserved as compared to LV (9). It remains possible, however, that this $NADP^+$-dependent activity may represent, at least in part,

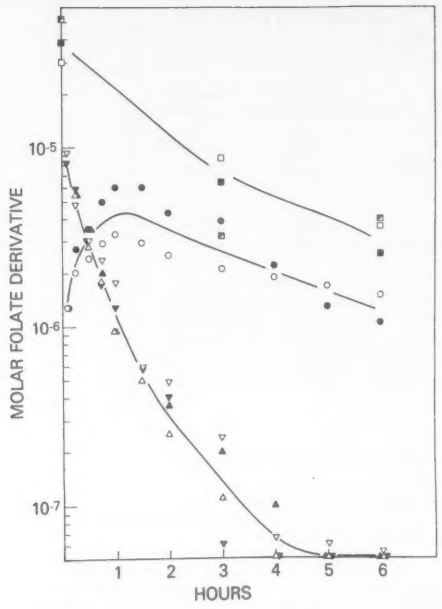

FIGURE 4.—Serum concentrations of LV, mTHFA, and MTX following an iv bolus of LV at the end of high-dose MTX therapy. Determinations of MTX levels were performed by Dr. C. E. Myers (2). LV levels were determined in 50-µl assays containing the components described in fig 2 as well as 5 µl of serum obtained at the indicated times (for early points 1 µl of serum with 4 µl of baseline serum was also used). mTHFA levels were determined in 50-µl assays containing the components described in fig 3 as well as 5 µl of serum obtained at the indicated times. Concentrations of LV and mTHFA derived from the standard curves were halved to give the concentrations of the active *l*-isomers. Patient 1 (osteosarcoma): ▲ = LV, ■ = MTX. Patient 2 (osteosarcoma): △ = LV, □ = MTX. Patient 3 (lymphoma): ▽ = LV, ○ = mTHFA, ◩ = MTX. Patient 4 (lymphoma): ▼ = LV, ● = mTHFA.

one or more other folate compounds derived from the infused LV. We have assumed that NADP$^+$-dependent activity in serum is representative of mTHFA for two reasons. First, mTHFA is the major folate derivative found in serum (3). Second, it has previously been demonstrated that iv doses of LV are substantially converted to mTHFA (9).

The preliminary pharmacokinetics of high-dose iv LV and its conversion to mTHFA as presented here agree with several studies in which substantially smaller doses of LV or mTHFA were administered as an iv bolus (9,15,17,18) and with one study in which a higher dose was administered as a prolonged iv infusion (19). These studies, although less detailed than the data presented here, also demonstrated rapid conversion of LV to mTHFA and a substantially longer half-life for mTHFA.

In conclusion, the assay for LV and mTHFA described here has allowed us to follow the kinetics of the rescue phase of high-dose MTX therapy and to obtain simultaneous levels of LV, mTHFA, and MTX. This ability may permit the design of more rational rescue regimens to minimize MTX toxicity and to avoid overtreatment with LV rescue. Such an approach may be particularly useful, since cytotoxicity in vitro from higher concentrations of MTX seems to be prevented only by use of at least equimolar concentrations of LV (1,20). In this regard, however, it should be noted that the serum MTX levels of our patients exceeded their serum levels of LV plus mTHFA for at least 6 hrs into the rescue period. They had no undue toxicity and were thus presumably adequately rescued.

REFERENCES

1. BERTINO JR. "Rescue" techniques in cancer chemotherapy: use of leucovorin and other rescue agents after methotrexate treatment. Semin Oncol 4:203–216, 1977.
2. MYERS CE, LIPPMAN ME, ELIOT HM, ET AL. Competitive protein binding assay for methotrexate. Proc Natl Acad Sci USA 72:3683–3686, 1975.
3. HERBERT V, LARABEE AR, and BUCHANAN JM. Studies on the identification of a folate compound of human serum. J Clin Invest 41:1134–1138, 1962.
4. ERBE RW, NAU MM, and LEDER P. Translation and translocation of defined RNA messengers. J Mol Biol 39:441–460, 1969.
5. BLAKLEY RC. The Biochemistry of Folic Acid and Related Pteridines. Amsterdam, The Netherlands, North-Holland Publishing Co, 1969, pp 188–218.
6. DICKERMAN HW, STEERS E, REDFIELD BG, ET AL. Methionyl soluble ribonucleic acid transformylase. I. Purification and partial characterization. J Biol Chem 424:1522–1525, 1968.
7. NAKAMOTO T, and KOLAKOFSKY D. A possible mechanism for initiation of protein synthesis. Proc Natl Acad Sci USA 55:606–613, 1966.
8. COSULICH DB, SMITH HM, and BROQUIST HP. Diastereoisomers of leucovorin. J Am Chem Soc 74:4215–4216, 1952.
9. NIXON PF, and BERTINO JR. Effective absorption and utilization of oral formyltetrahydrofolate in man. N Engl J Med 286:175–179, 1972.
10. MEHTA BM, GISOLFI AL, HUTCHINSON DJ, ET AL. Serum distribution of citrovorum factor and 5–methyltetrahydrofolate following oral and im administration of calcium leucovorin in normal adults. Cancer Treat Rep 62:345–350, 1978.
11. WATERS AH, and MOLIN DL. Studies on the folic acid activity of human serum. J Clin Pathol 14:335–342, 1961.
12. SPRAY GH. Microbiological assay of folic acid activity in human serum. J Clin Pathol 17:660–665, 1964.
13. ROTHENBERG SP, DACOSTA M, and ROSENBERG Z. A radioassay for serum folate: use of a two-phase sequential-incubation, ligand-binding system. N Engl J Med 286:1335–1339, 1972.

14. Raso V. Radioimmunoassays for methotrexate, citrovorum factor, neocarzinostatin, and actinomycin D. Cancer Treat Rep 61:585–590, 1977.
15. Rothenberg SP, DaCosta M, and Rosenberg Z. A radiochemical assay for N^5-formyltetrahydrofolic acid (citrovorum factor) in serum and urine. Anal Biochem 93:483–487, 1979.
16. Langone JJ, and Levine L. Immunoassay of leucovorin: use of ^{125}I–labeled protein A to detect immunological binding. Anal Biochem 95:472–478, 1979.
17. Spray GH, and Witts LJ. The utilization of folinic acid injected intravenously. Clin Sci 12:391–397, 1953.
18. Chanarin I, and Perry J. Metabolism of 5–methyltetrahydrofolate in pernicious anaemia. Br J Haematol 14:297–301, 1968.
19. Mehta BM, Shapiro WR, Rosen G, et al. Distribution of folates following methotrexate-leucovorin regimen in cancer patients. *In* Chemistry and Biology of Pteridines. Proceedings of the Sixth International Symposium on the Chemistry and Biology of Pteridines, La Jolla, CA, Sept 25–28, 1978 (Kisliuk RL, and Brown GM, eds). New York, NY, Elsevier North Holland, 1979, pp 677–682.
20. Pinedo HM, Zaharko DS, Bull JM, et al. The reversal of methotrexate toxicity to mouse bone marrow cells by leucovorin and nucleosides. Cancer Res 36:4418–4424, 1976.

Differential Pulse Polarographic Determination of BCNU Pharmacokinetics in Patients With Lung Cancer[1,2]

Raimondo Russo,[3] Ivan Bartošek,[3,*] Elena Piazza,[4] Anna Maria Santi,[3] Arnaldo Libretti,[4] and Silvio Garattini[3,5]

SUMMARY

BCNU levels were determined by differential pulse polarography in 12 patients with lung cancer. A simple assay method for submicrogram quantities of nitrosoureas is described. The half-life of BCNU added to plasma in vitro was 1413 mins at 0°C and 12 mins at 37°C during incubation. BCNU kinetics in blood of treated patients was calculated using the two-compartment open model; the half-life of the drug was 1.4 mins in the first phase and 17.8 mins in the second. Mean distribution volume was 2.59 liters/kg and plasma clearance was 16.7 ml/min/kg.

[Cancer Treat Rep 65:555–562, 1981]

Clinical use of nitrosoureas in single- and multiple-agent chemotherapy for various tumors has led to the development of new assay methods. Garrett and Cusimano (1) described the polarographic activity of various nitrosoureas in 1966. Polarographic techniques have been improved since then and their overall sensitivity has increased about 100-fold so that nitrosoureas can now be detected in biologic samples at below microgram levels (2).

Current methods of BCNU assay are based on spectrophotometry (3) (using Bratton-Marshall reaction, with an assay limit of 1 μg), on the use of labeled nitrosourea (4) (mostly limited to animal studies), and on chemical ionization mass spectrometry (5,6), with an assay limit of 50 ng of BCNU.

The main purpose of this paper is to present a simple procedure for differential pulse polarographic measurement of intact nitrosoureas in blood of patients with tumors. The low assay limit (20 ng) and the rapidity of the procedure make this an efficient and relatively inexpensive method for BCNU measurement in clinical laboratories. The in vitro experiments described in this paper showed it was essential to control the conditions of BCNU administration and the storage of plasma samples for nitrosourea assay.

Pharmacokinetics of BCNU was followed in 12 patients with lung cancer treated repeatedly according to two different schedules.

MATERIALS AND METHODS

Drugs and Reagents

BCNU (molecular weight, 214.04) used as polarographic standard, and its pharmaceutic form (Nitrumon, Simes, Milan, Italy) were of 99.8% purity. BCNU was stored at –20°C over a dessicator (silica gel).

All reagents (*n*-pentane, citric acid, sodium citrate, and KCl) were analytic grade. The batch of citric acid was checked for polarographic purity,

[1]Received Mar 10, 1980; revised Sept 29, 1980; accepted Oct 8, 1980.

[2]Supported by Italian National Research Council (CNR Contratto No. 79.00623.96), Control of Neoplastic Growth, and by Euratom contract 221–76–1 BIOI.

[3]Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

[4]Fifth Division of Medical Pathology, University of Milan, "Luigi Sacco" Hospital, Italy.

[5]We thank Dr. Stan Sýkora (Bruker Spektrospin Co, Milan, Italy) for his valuable advice and for building the special modules used for the "three-point" polarographic current readings, Dr. Vittorio Vecchietti (Simes Research Laboratories, Milan, Italy) for the generous supply of BCNU, and Mrs. Judy Baggott for her help and advice in the preparation of the manuscript.

*_Reprint requests to_: Ivan Bartošek, PhD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

since it had been noted that impurities giving waves in the neighborhood of the BCNU peak were present in some preparations. Tridistilled, commercially supplied mercury was used for the dropping mercury electrode. High-purity nitrogen gas was used for removal of oxygen from polarographed samples.

Solutions

All solutions were prepared with redistilled water: citric acid (0.2 M, pH 1.8, 42.03 g/1000 ml); trisodium citrate (3.8%, 130 mM, adjusted to pH 5.5 with citric acid); and saturated KCl solution for calomel electrode.

Apparatus

Differential pulse polarography was performed with the modular Bruker Universal Polarograph E 310 (Karlsruhe, West Germany), with a standard polarographic vessel (Metrohm E 607, Herisau, Switzerland). A three-electrode arrangement was composed of dropping mercury electrode, platinum auxiliary electrode, and saturated calomel electrode as reference. A particular module was developed in collaboration with Dr. S. Sýkora (Bruker Spektrospin Co, Milan, Italy) for "three-point" reading of polarographic currents at preset potentials. An X-Y recorder (Hewlett-Packard 7010A) was connected with the polarograph for plotting current-potential curves.

A rotating extractor (60 rpm) was used for plasma extraction with *n*-pentane to prevent formation of water-solvent emulsions.

BCNU Extraction

Immediately after blood samples were taken, they were mixed with anticoagulant (nine parts blood plus one part 3.8% sodium citrate), cooled, and centrifuged (10 minutes, 3000 × *g*; 4°C).

Plasma samples were either extracted immediately with *n*-pentane or rapidly frozen and stored at –80°C. Frozen samples were quickly thawed in a shaking water bath (37°C) and then held in an ice bath before extraction.

Cold plasma (1 ml) was mixed with an equal volume of 0.2 M citric acid and extracted twice with 5 ml of *n*-pentane for 10 mins using the slowly rotating extractor. The combined extracts were evaporated in a large test tube containing 10 ml of 0.2 M citric acid under a nitrogen stream in a water bath at 40°C (5). The extracted BCNU passed quantitatively into citric acid solution.

Polarographic Measurement of BCNU (2)

Oxygen dissolved in the sample was removed by bubbling nitrogen for about 5 mins. The following conditions were used for polarographic assay: differential pulse height ΔE = 100 mv; sweep interval, between −300 and −700 mv; sweep rate, −2 mv/sec; mercury flow rate, 1.2 mg/sec; mercury column height, 80 cm; and drop time given by an electronically controlled hammer, 2.5 secs. The potentials preset on the special module for three-point readings were −350 mv (min), −500 mv (peak), and −650 mv (maj). All polarograms were plotted by the X-Y recorder as control of current-potential curves. Repeated three-point readings increased the precision of the measurements; the error in determination of parallel samples did not exceed 3.3%. Recovery of BCNU added to plasma and extracted was 80% ± 4% compared to the direct assay of the compound dissolved in 0.2 M citric acid. The detection of BCNU was calculated to be about 20 ng. Calibration curves for BCNU were linear between 0.02 and 20 μg/ml using different scales of current sensitivity.

Calculations

BCNU Concentration

The net height of the polarographic peak of BCNU (fig 1) is proportional to the drug concentration in the sample. It can be calculated from the currents recorded at preset potentials (three-point assay). The lowest and highest potentials (−350 and −650 mv) are chosen symetrically around the peak potential (−500 mv). The distance of the potentials depends exclusively on experimental conditions (eg, type, concentration, pH of the basic electrolyte, inteferences, etc).

The total height of the peak of the sample (S), or blank (B) is calculated from the currents (I):

$$S \text{ or } B = I_{peak} - 0.5 \cdot (I_{min} + I_{maj}).$$

The net height (H) corresponds to the difference between sample and blank total heights: H = S – B.

Comparison of sample net height (H_s) with net height of reference (H_r) with a known concentration of nitrosourea (c_r) gives the concentration in the sample (c_s):

$$c_s = \frac{H_s \cdot c_r}{H_r}.$$

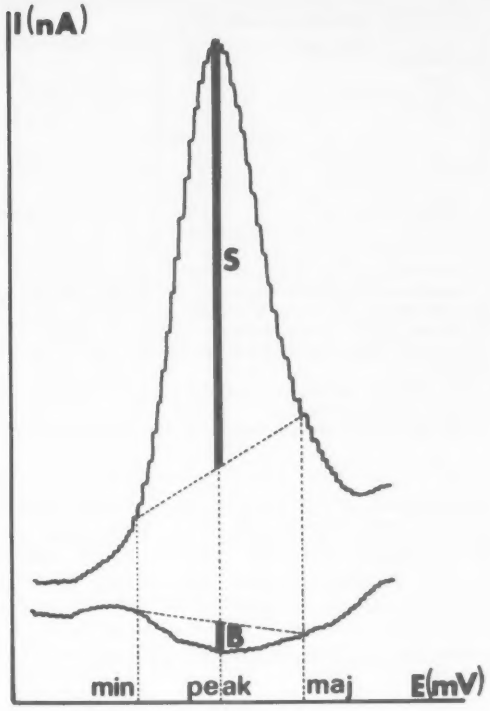

FIGURE 1.—Scheme of the 3-point reading of differential pulse polarographic currents. I = current (nAmpere); E = potential (mv); S = sample; B = blank; min, peak, and maj correspond to potentials – 350, – 500, and – 650 mv for BCNU.

BCNU Kinetics

BCNU kinetics in plasma in vitro was computed from the first-order equation:

$$c_t = c_0 \cdot e^{-\beta \cdot t} \quad t_{1/2} = \frac{\ln 2}{\beta}.$$

Disappearance of the drug from plasma of BCNU-treated patients was calculated using the method of residuals (7,8) from the two-compartment open-model equation:

$$c_t = c_{0\alpha} \cdot e^{-\alpha t} + c_{0\beta} \cdot e^{-\beta t}.$$

Results of infusion experiments were fitted into the equation:

$$c_t = \frac{I}{\beta \cdot V_d}(1 - e^{-\beta \cdot t}).$$

In these equations: c_0 and c_t are plasma concentrations of BCNU at the beginning ($t = 0$) and at time t; α and β are first-order rate constants of drug distribution and elimination; I is the constant infusion rate; and

$$V_d \text{ is the volume of distribution } V_d = \frac{\text{Dose}}{c_0}.$$

The area under the curve (AUC) was calculated using the trapezoidal rule:

$$AUC = 0.5 \cdot (c_0 t_0 + c_1 \cdot t_1 + c_2 \cdot t_2 + \ldots + c_n \cdot t_n).$$

Apparent plasma clearance was calculated from the formula:

$$CL_{pl} = \frac{\text{Dose}}{AUC \cdot \text{body weight}} \text{ (ml/min/kg)}.$$

Statistics

Curve fitting was performed by the least-squares method. Data were statistically analyzed using either Student's t test or Dunnett's test (9). Coefficients of variation were calculated for grouped parameters.

In Vitro Experiments

Batches of fresh, defibrinated, and heparinized blood containing BCNU (10 μg/ml) were incubated in thermostated water baths at 0°C, 20°C, and 37.5°C. One-milliliter samples were taken at intervals between 5 and 300 mins, centrifuged, and extracted for polarographic assay of BCNU decay. Similar experiments were performed after adding BCNU to solutions for iv infusion (5% glucose, 0.9% NaCl).

Patients

Ten males and two females with stage III TNM (10) inoperable lung cancer were chosen for BCNU treatments in a trial consisting of two schedules of antitumoral therapy (11). These patients were 49–73 years of age, weighed between 53 and 79 kg, and had a Karnofsky performance score > 5 (12). All patients had normal heart, kidney, and liver functions; a wbc count > 5000/mm³; a platelet count > 150,000/mm³, and blood hemoglobin > 9 g/100 ml. None of the patients had been treated previously with antitumoral drugs.

Six patients (five males and one female) were treated according to schedule A: BCNU at a total

dose of 50 mg iv; doxorubicin at a dose of 40 mg/m² iv; and cyclophosphamide at a dose of 600 mg/m² iv. The other six patients (five males and one female) were treated according to schedule B: BCNU at a total dose of 50 mg iv; vincristine at a total dose of 2 mg iv; and procarbazine at a dose of 100 mg/m²/day orally (Days 1–14). Schedules A and B were repeated in 28-day cycles. All patients were checked (hematocrit, platelet count, BUN, blood glucose, transaminase activities, and blood electrolytes) the day before starting each treatment cycle.

The pharmaceutic form of BCNU (Nitrumon, Simes) was dissolved in ethanol (100 mg of BCNU/3 ml), and sterile injectable water was added up to 30 ml while shaking. Fifteen milliliters of this solution containing 50 mg of BCNU was administered as an iv bolus during 1–2 mins. In two patients BCNU was infused by a peristaltic pump during 25–30 mins. The nitrosourea (50 mg) was dissolved with ascorbic acid (25 mg added to keep the pH between 4 and 5) in 100 ml of sterile glucose solution (5%).

In all treatments BCNU was administered before the other antitumoral drugs, in order to avoid any interference with polarographic assay of the nitrosourea.

Blood samples (5 ml) were taken from the noninjected arm of all patients at various intervals: between 2 and 240 mins after iv bolus, or during (5, 10, 15, and 25 mins) and at the end of BCNU infusion (between 2 and 10 mins after stopping infusion). The total number of monitored cycles was 24 with iv bolus administration and two with BCNU infusion.

RESULTS

In Vitro Experiments

BCNU was incubated with whole blood (10 μg/ml) at different temperatures at pH 7.4. The greater stability of the nitrosourea at low temperatures is evident from table 1. Generally, the half-life of the drug in blood can be correlated with the temperature by the equation:

$$\ln t_{1/2} = 7.161 - 0.128 \times (°C)$$

calculated from the same data (correlation coefficient $r = 0.997$).

Previous data from this laboratory (2) on the stability of BCNU in various solutions induced us to check decomposition of the drug added without acidification to the infused solution; about 93% of the nitrosourea disappeared within 15 mins at room temperature at pH 7.6 (unbuffered 0.9% NaCl in glass infusion vial) (fig 2). It has been noted that decomposition of BCNU differed in the various types of containers (glass; polyethylene) in which infusion solutions are supplied for clinical use. Addition of ascorbic acid (25 mg/100 ml of BCNU solution) was sufficient to keep the pH between 4 and 5.

TABLE 1.—BCNU* decomposition during incubation† with rat blood at different temperatures

Temperature (°C)	Half-life (mins)	r‡
0	1413.2 (1139.8–1859.1)§	0.923
22	61.3 (59.3–63.3)	0.998
37.5	11.9 (11.4–12.5)	0.994

*Initial concentration of BCNU was 10 μg/ml of blood.

†Samples of blood were taken at 0, 30, 60, 120, 180, and 300 mins during incubation at 0°C and 22°C and at 0, 5, 10, 15, 20, 30, 45, and 60 mins during incubation at 37.5°C. All samples were cooled, centrifuged, and acidified, and BCNU concentration was assayed.

‡Correlation coefficient r = the first-order rate of BCNU disappearance from rat blood.

§Values in parentheses = 95% confidence limits of half-life.

Patients

From the 24 therapeutic cycles monitored in 12 patients, seven could be evaluated only in the rapid distribution α-phase; α- and β-phases were both measured in 14 patients. The possible presence of a third, very slow phase (γ) was followed in two patients monitored for 4 hours. One cycle could not be evaluated because of the small number of blood samples.

Table 2 shows BCNU plasma levels between 2 and 60 mins in nine cycles monitored in four patients. Table 3 shows the respective pharmacokinetic parameters for the same patients. No significant trends could be found during repetition of treatment cycles in the same patients.

Table 4 summarizes the pharmacokinetic data derived from all BCNU assays in both groups treated according to schedules A and B. Again, no statistically significant difference was seen between the groups. Coefficients of variation for the initial concentrations of BCNU, depending on the rate of drug delivery during the iv bolus, were high (> 80%), and the same was true for the volume of distribution (> 50%). Coefficients of variation for

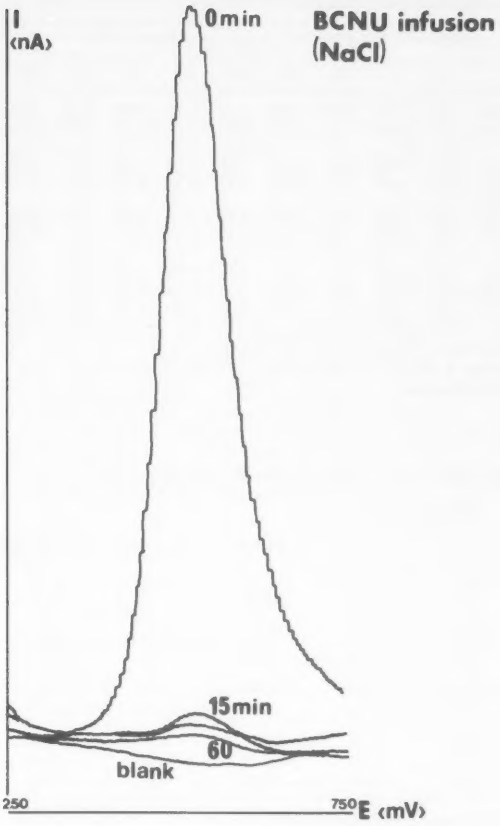

FIGURE 2.—Polarographic determination of BCNU decay in 0.9% NaCl, pH 7.6. I = current (nAmpere); E = potential (mv). Rapid decomposition of the nitrosourea at room temperature (20°C) is indicated by time elapsed after its addition to the infusion bottle with physiologic saline.

α- and β-half-lives, plasma clearance CL_{pl}, and AUC were much lower (between 37% and 40%).

When blood samples were taken at longer intervals after BCNU bolus (between 10 and 240 mins), a third γ phase could be documented with a half-life between 53 and 66 mins ($r = 0.9$ for the two patients examined).

BCNU levels were also assayed during and after iv infusion of the drug (fig 3). Maximum blood level of BCNU at the end of infusion was between 3.87 and 4.39 μg/ml; half-life of the nitrosourea after the end of infusion was between 4.0 and 4.2 mins (two patients).

DISCUSSION

Differential pulse polarographic determination was developed as a simple and selective assay of intact nitrosoureas in biologic samples (2). The presumed split products of BCNU, ie, dialkylazohydrozide and alkylisocyanate, are not measured by this method (13). The extraction procedure using *n*-pentane is suitable for lipid-soluble nitrosoureas (2) but in our experience does not extract water-soluble derivatives (streptozocin). On the other hand, this reduces the possible interferences. It is preferable to extract plasma rather than whole blood, because the lipids in cell membranes disturb polarographic measurements; electric current irregularities may be caused by interaction of tiny lipid drops with the surface of the dropping mercury electrode. The lipids, transferred into the basic electrolyte after evaporation of the *n*-pentane extract, can be removed simply by filtering the sample through analytic paper. However, in our experience, a similar procedure may be used for nitrosourea assay in more lipid-rich tissues, such as liver homogenate, without major difficulties.

In comparison with other methods these limitations are amply compensated by the simplicity of treatment and sample handling and by the high sensitivity (assay limit, 20 ng). Differential pulse polarography is thus a suitable technique for clinical measurement of nitrosourea levels in blood of treated patients.

Our initial aim was to establish the best conditions for handling and preserving blood and plasma samples without loss of BCNU before polarographic assay. We would recommend a rapid procedure at low temperatures (table 1). Moreover, attention must be paid to the pH in preparation of BCNU solutions for infusion (or iv bolus). Keeping the pH at about 5.5, nitrosoureas are stable (1,14). Apparent activation energies for first-order degradation were calculated for different temperatures and pH (14). Moreover, it was noted that the material the container is made of (glass or plastic) may influence the pH of the infusion solution (possible diffusion of CO_2 through plastic, or of alkaline ions from glass). Addition of ascorbic acid overcame the instability of BCNU during infusion.

BCNU degradation in blood determined by polarography (half-life, 11.9 mins) corresponded to the value established by chemical ionization mass spectrometry (half-life, 11.6 mins) (5). It is also interesting that nitrosourea decay is proportional to temperature [$\ln t_{1/2} = a + b \times (°C)$]; this means the possible decomposition rate of BCNU can be calculated in biologic samples during handling.

TABLE 2.—BCNU* plasma levels in patients with lung cancer

Patient No.	Age (yrs)/sex/wt (kg)	Schedule†	Treatment cycle	Bolus (secs)	BCNU (μg/ml of plasma) at following times (mins)‡— 2	3.5	5	8	12	15	20	30	45	60
1	55/M/56	A	1	60	ND§	5.48	3.60	2.00	1.30	1.10	0.70	0.60	0.30	ND
			2	75	10.96	4.78	2.91	1.58	1.22	0.85	0.68	0.51	0.25	ND
1	65/M/70	A	1	120	11.12	4.04	3.91	1.76	0.93	ND	0.54	0.45	0.30	0.23
			2	140	5.16	2.02	1.56	0.79	0.73	ND	0.52	0.33	0.21	0.07
3	71/M/64	B	4	120	12.20	ND	ND	1.07	0.52	0.45	ND	0.13	ND	0.01
			6	160	14.60	ND	ND	1.08	0.51	ND	0.37	ND	0.14	0.08
4	73/M/64	B	1	75	5.80	ND	2.00	1.50	1.00	ND	0.52	0.32	0.18	0.07
			2	100	10.40	3.70	1.65	0.87	0.70	ND	0.58	0.21	0.12	0.05
			3	60	7.10	4.38	3.40	1.75	1.00	ND	1.00	0.58	0.48	0.33

*BCNU was administered in an iv bolus of 50 mg/15 ml of injectable water during the period indicated.
†Treatment schedule: A (BCNU + doxorubicin + cyclophosphamide); B (BCNU + vincristine + procarbazine).
‡Blood samples were taken at intervals indicated from the contralateral arm.
§ND = not determined.

TABLE 3.—Pharmacokinetic parameters of BCNU* disappearance from plasma of patients with lung cancer

Patient No.	Age (yrs)/sex/wt (kg)	Schedule	Treatment cycle	Half-life (mins) α	β	AUC†	V_d (liters/kg)	CL_{pl} (ml/min/kg)
1	55/M/56	A	1	0.91	14.86	67.47	0.65	9.16
			2	1.08	14.83	59.08	1.58	15.11
2	65/M/70	A	1	2.06	31.49	62.76	3.22	11.38
			2	1.06	15.38	35.68	3.74	20.01
3	71/M/64	B	4	1.30	8.61	38.55	3.47	20.26
			6	1.34	17.92	66.39	1.14	11.76
4	73/M/65	B	1	1.06	12.63	42.20	3.27	17.79
			2	0.75	12.23	44.72	0.85	17.20
			3	1.71	25.80	65.89	3.85	11.67

*All parameters were calculated from the data given in table 2. Correlation coefficients were 0.9.
†AUC was calculated from BCNU concentrations using the trapezoidal rule. Initial concentration of BCNU was extrapolated from β place.

As results from comparison of the coefficients of variability for various pharmacokinetic parameters, the group of patients entering this study can be considered a homogeneous population. The high coefficient of variation found for the initial calculated BCNU concentration ($c_0\alpha$) depends mostly on the rate of drug delivery, so it is best to standardize the bolus time. The other pharmacokinetic parameters (α- and β-half-lives, plasma clearance CL_{pl}, and AUC), characterizing bioavailability of the drug, all depending on the organism's overall capacity to metabolize and eliminate BCNU, were much less dispersed. Distribution volume found in our experiments, 2.59 liters/kg, was close to that given by Levin et al (5) (3.25 liters/kg); plasma clearance of BCNU (16.6 ± 1.7 SE ml/min/kg) was within the limits given by the same authors (56 ± 56 SD ml/min/kg). In addition, the half-life values were close to the values reported in the literature (2,5).

A pilot study of BCNU levels in the blood of two patients followed for 4 hours after iv bolus indicated that probably a third, slow phase exists (half-life 50–60 mins); complementary observations are in progress. Levin et al (15) monitored patients for 30 hrs and found a rapid phase with a clearance half-life of 7 mins and a slow phase with a half-life of 4.6 hrs. The difference between their results and ours may be due to the length of the observation period (4 vs 30 hrs).

In general, our results with patients with lung cancer showed no differences between the two treatment schedules, between single and repeated cycles of BCNU administration, or between measurements within individuals.

TABLE 4.—Pharmacokinetic parameters of BCNU disappearance from plasma: comparison of treatment schedules A and B*

	Co† (μg/ml)	Half-life (mins)‡ α	Half-life (mins)‡ β	AUC§ (α + β)	V_d (liter/kg)	CL_{tot} (ml/min/kg)
			Schedule A			
Mean	24.77	1.45	18.06	44.45	2.32	14.70
SE	± 6.51	± 0.15	± 3.40	± 7.70	± 0.55	± 2.00
n¶	10	10	6	6	6	6
			Schedule B			
Mean	20.14	1.43	17.64	38.24	2.76	17.94
SE	± 5.15	± 0.15	± 2.45	± 4.56	± 0.52	± 2.49
n	11	11	8	8	8	8
			Schedules A + B			
Mean	22.31	1.44	17.82	40.90	2.59	16.69
SE	± 4.38	± 0.12	± 1.94	± 4.16	± 0.37	± 1.71
n	21	21	14	14	14	14

*Treatment schedule: A (BCNU, 50 mg iv + doxorubicin, 40 mg/m² iv + cyclophosphamide, 600 mg/m² iv); B (BCNU, 50 mg iv + vincristine, 2 mg iv + procarbazine, 100 mg/m² orally).

†Co = calculated initial concentration of BCNU (μg/ml of plasma).

‡2-compartment open model was used for calculation of α- and β-half-lives. All correlation coefficients were > 0.9.

§AUC was calculated by trapezoidal rule as index of BCNU bioavailability.

¶*n* = No. of trials used for calculations.

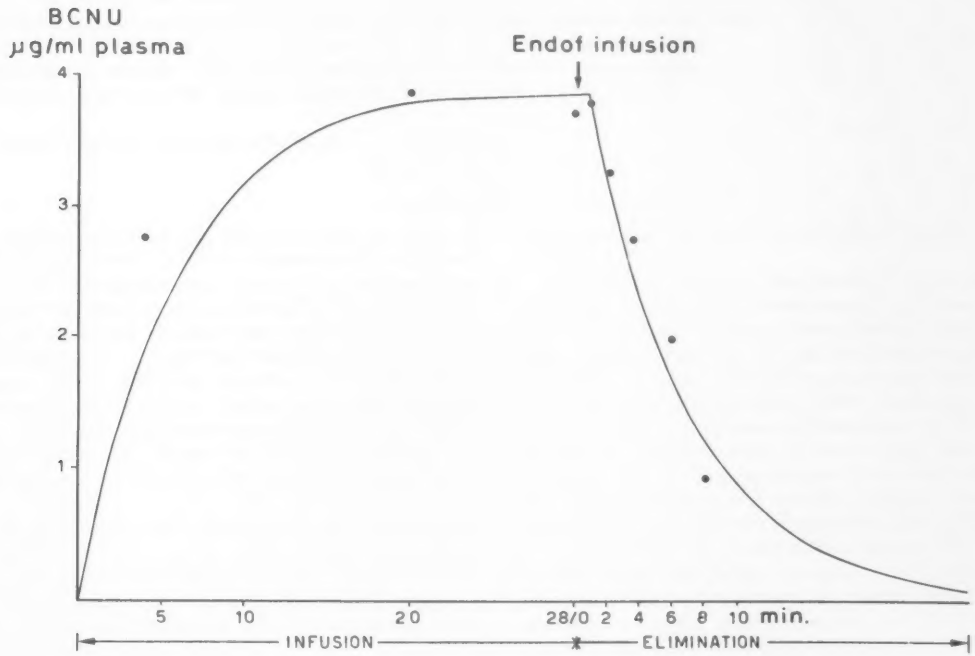

FIGURE 3.—BCNU concentration in plasma during infusion. Patient is 49 yrs old, weighs 69 kg, and is receiving treatment schedule B (3rd cycle). BCNU (50 mg) was infused during 28 mins (infusion rate, I = 1785.7 μg/min; apparent distribution volume, V_d = 2757.1 ml; half-life calculated for the elimination phase half-life = 4.18 mins, ie, β = − 0.1657 min^{-1}.

REFERENCES

1. Garrett ER, and Cusimano AG. Polarography of various *N*-alkyl-*N*-nitrosoureas. J Pharm Sci 55:702–710, 1966.
2. Bartosek I, Daniel S, and Sýkora S. Differential pulse polarographic determination of submicrogram quantities of carmustine and related compounds in biological samples. J Pharm Sci 67:1160–1163, 1978.
3. Loo TL, and Dion RL. Colorimetric method for the determination of 1,3-bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 54:809–810, 1965.
4. DeVita VT, Denham C, Davidson JD, et al. The physiological disposition of the carcinostatic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clin Pharmacol Ther 8:566–577, 1967.
5. Levin VA, Hoffman W, and Weinkam RJ. Pharmacokinetics of BCNU in man: a preliminary study of 20 patients. Cancer Treat Rep 62:1305–1312, 1978.
6. Weinkam RJ, Wen JHC, Furst DE, et al. Analysis for 1,3-bis(2-chloroethyl)-1-nitrosourea by chemical ionization mass spectrometry. Clin Chem 24:45–49, 1978.
7. Rescigno A, and Segre G. La Cinetica dei Farmaci e dei Traccianti Radioattivi. Torino, Boringhieri, 1961.
8. Notari RE, and De Young. Biopharmaceutics and Pharmacokinetics. New York, NY, M. Dekker, 1975.
9. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 50:1096–1121, 1955.
10. International Union Against Cancer. TNM: Classification of Malignant Tumours, 2nd ed, Geneva, Switzerland, UICC, 1974.
11. Eridani S, Libretti A, Marsoni S, et al. Polychemotherapeutic strategy for inoperable bronchogenic carcinoma. *In* Proceedings of the 12th International Congress of Cancer, Buenos Aires, Argentina, Oct 5–11, 1978. (Abstr 8).
12. Livingston RB, Fee WH, Einhorn LH, et al. BACON (bleomycin, adriamycin, CCNU, Oncovin and nitrogen mustard) in squamous lung cancer. Experience in fifty patients. Cancer 37:1237–1242, 1976.
13. Oliverio VT, Kohn KW, and Woolley PW. Studies on the pharmacology and mode of action of the nitrosoureas. *In* Proceedings of the 11th International Cancer Congress, Florence, Italy (Oct 20–26, 1974): Surgery, Radiotherapy and Chemotherapy of Cancer (Bucalossi P, Veronesi U, and Cascinelli N, eds). Amsterdam, The Netherlands, Excerpta Medica, 1975, vol 5, pp 238–247.
14. Laskar PA, and Ayres JW. Degradation of carmustine in acqueous media. J Pharm Sci 66:1073–1076, 1977.
15. Levin VA, Weinkam RJ, Hoffman W, et al. Pharmacokinetics of BCNU in humans. Proc Am Assoc Cancer Res and ASCO 18:76, 1977.

Adrenal Response to Serial Cosyntropin Stimulation After Repeated High-Dose Prednisone Administration in Patients With Lymphoma[1,2]

Kenneth Zamkoff,* Jeffrey Kirshner, Dianne Cass, and Myron Miller[3,4]

SUMMARY

To determine if repeated courses of high-dose prednisone given to patients with lymphoma as part of multiple-drug chemotherapy programs would lead to progressive adrenal suppression, serial cosyntropin stimulation tests were performed. Four patients with diffuse histiocytic lymphoma (group 1) received prednisone for 5 days every 3 weeks for five courses, and five patients with Hodgkin's disease (group 2) received prednisone for 14 days every 4 weeks for six courses. Testing was done on Day 1 of each treatment course prior to the administration of therapy and after the final course of chemotherapy. In group 1 patients, there was no evidence of adrenal suppression after any of the courses of prednisone. The plasma cortisol increments after cosyntropin injection were also normal. In the group 2 patients, significant depression of basal plasma cortisol concentrations was observed after the first and fifth courses of prednisone, compared to the pretreatment values. The depression reflected the previous course of prednisone administration only and was not progressive with subsequent courses. The plasma cortisol increments after cosyntropin injection were normal despite depressed basal plasma cortisol levels.

[Cancer Treat Rep 65:563–566, 1981]

High-dose prednisone administration (ie, doses of 40–60 mg/m^2 daily in divided doses) is routinely used as part of multiple-drug therapy in the treatment of both Hodgkin's and non-Hodgkin's lymphoma (1). Most treatment programs use repeated courses of prednisone at regular intervals for periods of 5–14 days. When prednisone is given in doses of ≤ 40 mg once daily for 5–7 days, it does not appear to result in significant hypothalamic-pituitary-adrenal (HPA) suppression (2). However, impaired adrenal responses to ACTH infusion have been reported in patients receiving 20–30 mg of prednisone daily for 5–13 days (3).

Most studies that have been done to characterize HPA suppression in patients receiving glucocorticoids as part of a cancer chemotherapy program have examined the time course of adrenal suppression after a single course of glucocorticoid therapy (4,5). A study of 14 patients with lymphoma and multiple myeloma showed a diminished ACTH response to insulin-induced hypoglycemia with increasing numbers of prednisolone treatment courses (6). Cortisol responses to hypoglycemia and cosyntropin were near normal, suggesting that with repeated courses of prednisolone, suppression occurred at the HP level. However, there was no mention of the exact number of courses of steroid therapy, the doses of prednisolone used, or which course of steroid therapy preceded study of the patients.

In the present study, we attempted to determine if repeated courses of high-dose prednisone therapy, given as part of a cancer chemotherapy pro-

[1]Received May 7, 1980; revised Sept 9, 1980; accepted Sept 18, 1980.

[2]Supported by funds from the Veterans Administration Research Service.

[3]Medical Service, Veterans Administration Medical Center and the Department of Medicine, State University of New York, Upstate Medical Center, Syracuse.

[4]We thank Mrs. Kathy Neuner who carried out the cortisol determinations.

*_Reprint requests to:_ Kenneth Zamkoff, MD, Department of Medicine, Section of Hematology, Upstate Medical Center, 750 E Adams St, Syracuse, NY 13210.

gram, would result in significant and progressive adrenal hyporesponsiveness with successive courses of glucocorticoid administration.

METHODS

Four patients with diffuse histiocytic lymphoma (group 1) and five with Hodgkin's disease (group 2), who were to receive repeated courses of oral prednisone as part of their chemotherapy, were studied. The ages ranged from 21 to 71 years. No patient received radiotherapy. Group 1 patients were treated with prednisone (100 mg daily in three or four divided doses for 5 days every 3 weeks for five courses) combined with cyclophosphamide (750 mg/m^2 iv), doxorubicin (50 mg/m^2 iv), and vincristine (2 mg iv), on Day 1 of therapy. In the group 1 patients, bleomycin was also given (2 units/day as a continuous iv infusion for 5 days, for the first three courses). Group 2 patients received prednisone (60–80 mg daily in three or four divided doses for 14 days every 4 weeks for six courses), combined with mechlorethamine (6 mg/m^2 iv on Days 1 and 8), vinblastine (4 mg/m^2 iv on Days 1 and 8), and procarbazine (100 mg/m^2 orally for 14 days). Each course of prednisone was stopped abruptly in both groups.

To assess adrenal responsiveness, cosyntropin (0.25 mg) was administered iv. Plasma cortisol levels were measured just prior to cosyntropin administration and 30 and 60 minutes later. Cortisol determinations were done by a fluorescence procedure using a Turner fluorometer (7). The cosyntropin tests were performed prior to the administration of chemotherapy on Day 1 of each course of chemotherapy. An additional cosyntropin test was done in all patients at the same time interval as the previous tests, at the completion of either five or six courses of chemotherapy. The tests were done between 7:30 and 9:00 AM.

RESULTS

The mean times between the end of a course of prednisone and the next cosyntropin test were 20.1 ± 1.9 (SD) days for group 1 and 14.1 ± 0.9 days for group 2. In the group 1 patients, there were no significant differences in the mean basal plasma cortisol concentrations and the mean pretreatment basal plasma cortisol levels after any of the five courses of therapy (table 1). Likewise, in the group 1 patients, there were no significant differences in the mean 30- or 60-minute increments in plasma cortisol concentration after any of the five courses of therapy, compared to the mean 30- and 60-minute increments determined prior to therapy (table 1). The plasma cortisol responses to cosyntropin administration were considered normal after each performance of the test (4,8).

In the group 2 patients there were significant decreases in the mean basal plasma cortisol concentrations after the first and fifth courses of prednisone, compared to the pretreatment mean basal cortisol levels (23.2 vs 15.1 vs 10.5 μg/dl, respectively) (table 2). The mean increments in plasma cortisol concentrations at 30 and 60 minutes after cosyntropin injection were greater at both 30 and 60 minutes after each course of prednisone than prior to therapy, which may reflect the high pretreatment basal cortisol levels, but these differences were not statistically greater than in the control group.

DISCUSSION

Glucocorticoids are now routinely used as part of multiple-drug chemotherapy programs for both Hodgkin's and non-Hodgkin's lymphomas. The emphasis toward aggressive initial therapies aimed at improved complete response rates in the prognostically unfavorable lymphomas has meant accepting significant hematologic toxicity. In addition to the

TABLE 1.—Cortisol levels in group 1 patients treated with prednisone*

Treatment course No.	Cortisol level		
	Basal (μg/dl)	Increments after 30 mins (μg/dl)	Increments after 60 mins (μg/dl)
Pretreatment	16.3 ± 4.2	14.6 ± 4.7	16.7 ± 3.8
1	15.7 ± 1.6	10.9 ± 5.7	15.0 ± 5.5
2	13.3 ± 0.7	12.8 ± 7.0	22.6 ± 8.1
3	13.6 ± 2.3	15.9 ± 2.6	19.2 ± 4.5
4	14.9 ± 5.2	15.0 ± 2.8	19.8 ± 2.7
5	16.8 ± 6.6	15.6 ± 4.7	21.0 ± 3.1

*4 patients received prednisone (100 mg/day × 5 q3wks); cortisol levels were determined following cosyntropin injection; values = mean ± SD.

TABLE 2.—Cortisol levels in group 2 patients treated with prednisone*

Treatment course No.	Cortisol level		
	Basal (μg/dl)	Increments after 30 mins (μg/dl)	Increments after 60 mins (μg/dl)
Pretreatment	23.2 ± 5.1	9.7 ± 4.4	11.9 ± 5.8
1	15.1 ± 4.3†	14.9 ± 4.0	19.5 ± 5.3
2	14.0 ± 6.3	10.5 ± 5.2	31.6 ± 19.7
3	14.6 ± 5.0	14.8 ± 5.4	24.1 ± 11.1
4	12.3 ± 5.3	16.3 ± 5.5	20.2 ± 6.5
5	10.5 ± 2.5*	17.2 ± 1.9	24.0 ± 5.4
6	15.7 ± 4.9	13.5 ± 2.9	16.4 ± 4.2

*5 patients received prednisone (60–80 mg/day × 14 days q4wks); cortisol levels were determined following cosyntropin injection; values = mean ± SD.

†$P < 0.05$ (paired t test), comparing the mean value following a given treatment course to the pretreatment mean.

risks of intercurrent illness due to bleeding from thrombocytopenia or severe infection from neutropenia, the question of adrenal suppression arises in relation to patients recently treated with high-dose glucocorticoid therapy. As a result, the patients are often "covered" with steroids during an acute illness. As most treatment programs are administered on a recurrent schedule, the question of glucocorticoid-induced HPA suppression frequently arises. Furthermore, the question of whether repeated administration of high-dose glucocorticoid therapy results in progressive HPA suppression has not been adequately addressed.

During recovery from exogenous glucocorticoid-induced HPA suppression, the adrenal gland lags behind the HP axis in its ability to respond to stress or insulin-induced hypoglycemia (9,10). Thus, the assumption is made that complete HPA recovery has occurred when there is a normal cortisol response to exogenously administered ACTH (2,3,11). Synthetic cosyntropin administration is equivalent to either insulin-induced hypoglycemia or surgical stress in eliciting maximal cortisol output by the adrenal gland (4,11).

When prednisone was administered at doses of 25 mg twice a day for 5 days, peak plasma cortisol concentrations and incremental cortisol levels remained depressed, compared to pretreatment values, following a cosyntropin test but not after an insulin tolerance test, when both were performed 5 days after the discontinuation of prednisone therapy. This suggested an intact HP response to stress but continued adrenal gland hyporesponsiveness (12). In 14 patients treated for a variety of hematologic malignancies with a single course of prednisone for 1, 2, or 4 weeks in doses equivalent to those our patients received, some patients in each group showed diminished adrenal response to cosyntropin stimulation for > 7 days after discontinuation of therapy (4). Most of the patients in each group had normal cosyntropin responses by Day 7. The six cancer patients who received iv methylprednisolone (125 mg once daily for 28 days) had normal AM and PM plasma cortisol levels and normal metyrapone responses on the day following the last day of therapy (5).

The present study indicates that in patients who receive high-dose prednisone therapy (HDPT) in divided doses for 5 days every 3 weeks for five courses, there is no adrenal suppression 3 weeks after the discontinuation of HDPT, as shown by cosyntropin tests of adrenal function. In patients who received 14 days of HDPT for five courses and who were tested 14 days after therapy was discontinued, the basal plasma cortisol concentrations tended to be lower and the increments in cortisol concentrations tended to be higher than the pretreatment values. The significant decrease in the basal cortisol level seen after the first course of prednisone may be spurious due to the high pretreatment basal cortisol level to which it was compared. These trends suggest modest basal adrenal suppression in the group 2 patients but a normal ability to respond to maximal stimulation. The increments in plasma cortisol concentrations also failed to show a delayed increase: the values 30 and 60 minutes after cosyntropin injection were normal. Similarly, with repeated courses of HDPT, the group 2 patients did not demonstrate progressive decreases either in basal plasma cortisol levels or in values 30 or 60 minutes after cosyntropin injection. Thus, the modest suppression in basal cortisol levels observed in the group 2 patients appears to represent suppression induced only by the previous course of HDPT.

The available literature suggests significant ad-

renal, as opposed to HP, suppression for periods of 5–7 days in patients treated with daily divided doses of HDPT for single courses lasting from 5 days to 4 weeks. By 14 days after the discontinuation of HDPT, however, adrenal responsiveness appears normal in patients given HDPT for 2 weeks. Presumably, the adrenal response of patients given courses of HDPT for < 2 weeks would also be normal at 14 days. There appears to be no cumulative adrenal suppression due to repeated courses of HDPT, indicating that the 14–20 days without steroid treatment was sufficient to allow for recovery of normal HPA function. Thus, the presence of normal basal cortisol levels indicating adequate basal hypothalamic-pituitary function, in conjunction with the normal adrenal responses to cosyntropin stimulation seen in the study, appears to obviate the need for steroid coverage in patients with lymphoma treated with repeated courses of HDPT of 5–14 days who have acute intercurrent illness occurring at least 2 weeks after the prednisone therapy has been discontinued. Adrenal insufficiency occurring in this setting suggests something other than steroid-induced adrenal suppression.

REFERENCES

1. Lewis BJ, and DeVita VT. Combination therapy of the lymphomas. Semin Hematol 15:431–457, 1978.
2. Byyny RL. Withdrawal from glucocorticoid therapy. N Engl J Med 295:30–32, 1976.
3. Axelrod L. Glucocorticoid therapy. Medicine 55:39–65, 1976.
4. Spiegel RJ, Oliff AI, Bruton J, et al. Adrenal suppression after short-term corticosteroid therapy. Lancet I:630–633, 1979.
5. Levitt M, Sharma RN, and Faiman C. Normal metyrapone response after 1 month of high-dose methylprednisolone in cancer patients: a phase I study. Cancer Treat Rep 63:1327–1330, 1979.
6. Wilson KS, Gray CE, and Cameron EHD. Hypothalamic-pituitary-adrenal function in patients treated with intermittent high dose prednisolone and cytotoxic chemotherapy. Lancet I:610–612, 1976.
7. Mattingly D. A simple fluorometric method for the estimation of free 11-hydroxycorticoids in human plasma. J Clin Pathol 15:374–379, 1962.
8. Graber AL, Ney RL, and Nicholson WE. Natural history of pituitary adrenal recovery following long term suppression with corticosteroids. J Clin Endocrinol Metab 25:11–16, 1965.
9. Wood JB, Frankland AW, James VHT, et al. A rapid test of adrenocortical function. Lancet I:243–245, 1965.
10. Donald RA, and Espiner EA. The plasma cortisol and corticotropin response to hypoglycemia following adrenal steroid and ACTH administration. J Clin Endocrinol Metab 41:1–6, 1975.
11. Kehlet H, and Binder C. Value of an ACTH test in assessing hypothalamic-pituitary-adrenocortical function in glucocorticoid treated patients. Br Med J 2:147–149, 1973.
12. Streck WF, and Lockwood DH. Pituitary adrenal recovery following short term suppression with corticosteroids. Am J Med 66:910–914, 1979.

Enhanced Cytostatic Effectiveness of Aniline Mustard Against 7,12-Dimethylbenz[*a*]anthracene-Induced Rat Mammary Tumors During Regression in Response to Ovariectomy [1]

Catherine Benckhuysen,* Hennie G. J. Ter Hart, and Pim J. Van Dijk [2]

SUMMARY

Sprague-Dawley rats bearing 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors were treated with either of two aromatic alkylating agents, aniline mustard or melphalan, alone or combined with ovariectomy. Both drugs were applied once a week for 8 weeks. Eighty-four percent of the tumors responded to ovariectomy, 38% regressing completely and 46% regressing partially. Aniline mustard, though virtually ineffective as a single agent, appeared synergistic with ovariectomy: a 100% regression rate (72% complete, 28% partial) was observed for this combination. Treatment with melphalan was as effective as ovariectomy, but the combination of melphalan with ovariectomy was no more effective than either treatment alone. The end product of aniline mustard metabolism, *p*-hydroxyaniline mustard *O*-glucuronide, may be more extensively activated by β-glucuronidase in hormonally regressing than in growing or stationary tumors. Intratumoral levels of β-glucuronidase occurring in DMBA-induced tumors 4 days after ovariectomy were found to be similar to those in the aniline mustard-sensitive mouse plasma cell tumor ADJ/PC6. It remains to be more extensively studied whether an effect of endocrine treatment on tumor β-glucuronidase levels, and possibly on intracellular distribution of enzyme, could be used therapeutically. An effectively scheduled cytostatic treatment (with a drug conjugate such as that formed metabolically from aniline mustard) in conjunction with ovariectomy might be effective in the treatment of hormone-responsive breast cancer.

[Cancer Treat Rep 65:567–574, 1981]

Aniline mustard, one of the earliest aromatic nitrogen mustards (1), has been shown to be metabolized in the liver. It is oxidized to *p*-hydroxyaniline mustard, which is then converted to an *O*-glucuronide[3] (2). *p*-Hydroxyaniline mustard was more active than aniline mustard against ADJ/PC5 plasma cell tumor in mice (3) and against Walker 256 carcinosarcoma in rats (4). It is, however, also a much more toxic compound in vivo. Synthetic *O*-glucuronide of *p*-hydroxyaniline mustard was found to be considerably less toxic to mice than unconjugated *p*-hydroxyaniline mustard (5). Since the glucuronide can serve as a substrate for purified β-glucuronidase (E. C. 3.2.1.31) (6), *p*-hydroxyaniline mustard can be expected to be liberated in vivo from its glucuronide in tissues with a high level of this enzyme. A striking example is the ADJ/PC5, or the related PC6, plasma cell tumor that is assumed to be sensitive to both aniline mustard (7) and *p*-hydroxyaniline mustard *O*-glucuronide (5) because of its high level of β-glucuronidase. One more transplantable tumor has been found in mice that, for a similar reason, seemed to be sensitive to treatment with

[1]Received June 5, 1979; revised Aug 13, 1980; accepted Oct 8, 1980.

[2]Division of Experimental Chemotherapy and Radiotherapy, Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Institute, Amsterdam.

[3](*p*-*N*,*N*-Di-[2-chloroethylamino]phenyl-β-D-glucopyranosid)-uronic acid.

**Reprint requests to:* Catherine Benckhuysen, MD, Department of Biochemistry, Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands.

aniline mustard (8). A partial correlation appeared to exist between histochemical glucuronidase staining and tumor regression in a phase II clinical trial on aniline mustard (9).

It has been reported that hormone-sensitive rat mammary tumors respond to endocrine ablation with a marked increase in contents of several lysosomal enzymes, among which is β-glucuronidase (10–14). Such an increase also occurs in lactating mammary gland in response to enforced cessation of lactation (15–17). These phenomena led us to study the cytotoxic effectiveness of aniline mustard during tumor regression following ovariectomy. Melphalan, which differs from aniline mustard in that it is not known to be metabolized to a glucuronide, was studied for comparison. 7,12-Dimethylbenz[*a*]anthracene (DMBA)-induced hormone-responsive rat mammary tumors were used. Like hormone-responsive human breast cancer, they are sensitive to ovariectomy and to treatment with steroid hormones or steroid hormone analogs but they are not very sensitive to cytostatic treatments (18,19).

MATERIALS AND METHODS

Tumors.—Forty-two female inbred Sprague-Dawley rats bearing DMBA-induced mammary tumors were selected for the experiment. Tumors had been induced by intubation of 20 mg of DMBA in 1 ml of olive oil at 50 ± 3 days after birth. Rats were assigned to six experimental groups on Day 0, which was 28–42 days after they had developed at least one tumor of 1-cm average diameter. Rats without tumor 120 days after receiving DMBA were discarded to ensure that a minimum of hormone-unresponsive tumors would be involved at the beginning of the experiment. Rats were not used if their tumors had shown spontaneous regression before Day 0. Three of 89 tumors grew rapidly to > 10 g during the observation period. These tumors were surgically removed under aseptic conditions well before Day 0. Wound healing offered no problems. At Day 0 the average tumor mass per rat was 3.21 ± 2.32 g, and the average tumor number was 2.05; 124 ± 17 days had passed since DMBA intubation.

Tumor growth and response.—Caliper measurements of two perpendicular axes, one across the longest dimension, were made twice weekly from 1 month after DMBA intubation until 60 days after the beginning of treatment. Thereafter, measurements were made once a week. Tumor masses were calculated using the formula $(\text{length} \times \text{width})^2/2$. Palpations were performed under ether anesthesia. Tumors were designated completely regressed when at least two successive palpations were negative. A > 50% decrease in mass of a tumor was designated a partial response; a decrease of < 50% was designated no response.

Treatments.—Ovariectomies were performed on Day 0; cytostatic treatment or vehicle control intubation was started on Day 4. Eight weekly doses of aniline mustard (40 mg/kg) or melphalan (10 mg/kg) were administered in 1 ml of olive oil. Rats were observed until Day 120. Aniline mustard was synthesized according to a modification of the procedure in (1). A commercial preparation of melphalan was used.

Sampling of tumor tissues.—Immediately after tumor excision, healthy-looking 0.1–0.5-g samples were rapidly frozen by submersion in isopentane at about –150°C and stored at –70°C. The series involved seven tumors in four intact rats and five tumors in five rats, four tumors in three rats, three tumors in three rats, and three tumors in one rat sampled at 4, 7, 14, or 28 days postovariectomy, respectively.

Tissue preparation.—With the aid of a dismembrator, frozen tumor samples were reduced to a powder during 1 min in a capsule cooled in liquid nitrogen. The pulverized tissue was suspended in 20 volumes of 0.9% NaCl in 0.02 M imidazol-HCl (pH 7.4) containing 0.05% Triton X-100 and was homogenized in a Potter-Elvehjem homogenizer. After centrifugation for 10 min at 700 × *g*, the supernatant was diluted with bovine serum albumin (BSA) in 0.02 M imidazol-HCl (pH 7.4) to a final concentration of 0.1% BSA.

Enzyme assay.—β-Glucuronidase activity was determined with the substrate 4-methylumbelliferyl-β-D-glucuronide according to a method slightly modified from Mead et al (20). A saturated solution of digitonin (5 μl) was added for solubilization of the enzyme; 0.1% BSA was required to prevent dissociation of the enzyme into its subunits. Enzyme activities were expressed in units per milligram of tumor protein. Protein was measured by the method of Lowry et al (21).

RESULTS

The effect of treatment with aniline mustard or melphalan on DMBA-induced rat mammary tumors was studied in groups of seven rats, either intact or subjected to ovariectomy 4 days before the beginning of cytostatic treatment.

Drug Tolerance

In preliminary studies on drug tolerance, groups of five or six normal rats survived at least 5 weeks after treatment with eight weekly oral doses of 40,

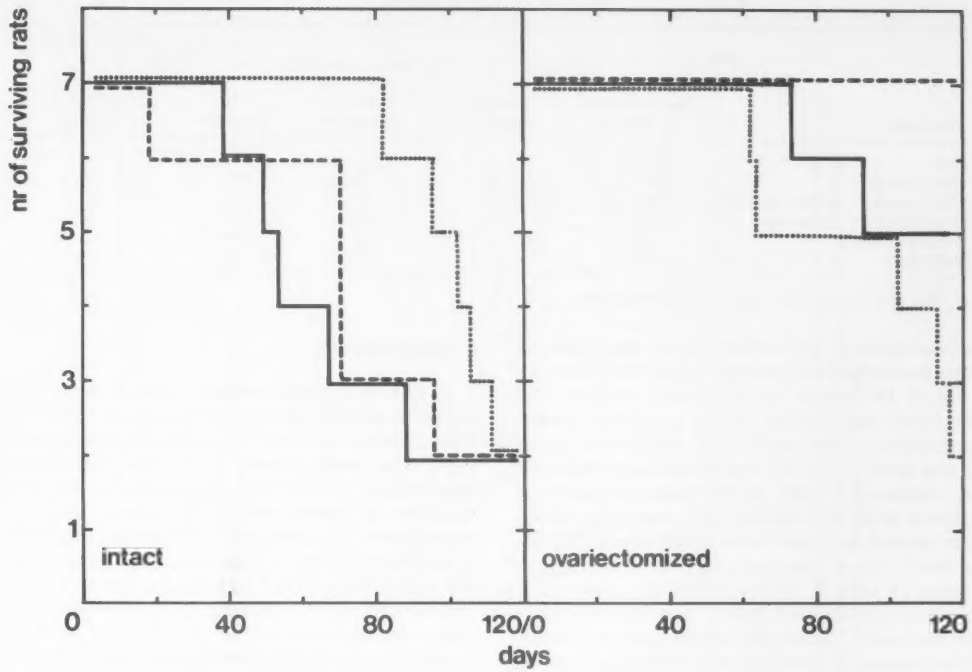

FIGURE 1.—Cumulative mortality of control and cytostatically treated intact or ovariectomized Sprague-Dawley rats bearing DMBA-induced mammary tumors. Ovariectomy at Day 0. _____ = No chemotherapy; —— = aniline mustard, every 7 days × 8, started on Day 4; and ..... = melphalan, every 7 days × 8, started on Day 4.

50, or 55 mg/kg of aniline mustard. However, a single dose of 60 mg/kg was lethal to two of two animals. Melphalan once a week at 10 or 12 mg/kg, but not 15 mg/kg, was nonlethal. It was tentatively concluded that cytostatic treatment with aniline mustard at 40 mg/kg/week × 8 was approximately equitoxic to 10 mg/kg of melphalan at a similar schedule. Both drugs were administered orally; melphalan has been reported to be tolerated markedly better when given orally rather than ip in various strains of rats (22).

Mortality Data

The cumulative mortality in the various groups is presented in figure 1. In the groups treated with aniline mustard, no mortality was observed among the ovariectomized rats, but for one intact animal that died shortly after the end of treatment, drug toxicity appears to have interfered. The death at Day 18 of a rat in the aniline mustard-treated group was apparently attributable to tumor growth. Melphalan treatment led to one toxic death among the intact rats and one among the ovariectomized rats. Toxic deaths have been excluded from calculation of regression data.

Response to Treatment

In table 1, responses obtained on the various treatment regimens are presented in terms of complete or partial regression of individual tumors and duration of complete remission. For a reason noninherent to the experiment, the observation period had to be restricted to 120 days; by then the median duration of complete remission was evaluable for all pertinent groups. Microscopy revealed that none of the nonrecurring tumors had been eradicated. If a late-occurring reduction in tumor volume was thought to be due to the rapid growth of a large tumor at another site in the host, the tumor was not considered therapeutically regressing.

Evaluation of the best-responding groups at Day 60, 1 week after the last dose of drug, revealed the following decreases in mass and number of tumors as compared to Day 0. In the seven rats treated

TABLE 1.—Tumor regression

Treatment	No. of tumors at Day 0*	% of tumors showing—			Median duration of complete remission (days)
		Complete regression	Partial regression	No response	
None	18 (7)	0	10	90	—
Ovariectomy	13 (7)	38	46	16	60
Ovariectomy + aniline mustard	18 (7)	72	28	0	77
Ovariectomy + melphalan	14 (6)	29	36	35	45
Aniline mustard	9 (6)	0	11	89	—
Melphalan	10 (6)	30	50	20	60

*No. of evaluable rats is shown in parentheses.

with ovariectomy, 68% of the tumor mass and ten of 13 tumors remained, compared to 3% of the mass and six of 18 tumors in seven rats treated with ovariectomy plus aniline mustard. After ovariectomy combined with melphalan, 73% of the tumor mass and 12 of 14 tumors remained (in six evaluable rats), compared to 59% of the mass and seven of ten tumors in six rats treated with melphalan alone. In four control rats that were evaluable at Day 60, mean total tumor mass had increased to 690% of the mass at Day 0; in five evaluable rats in the aniline mustard-treated group, the corresponding figure was 350%. Although aniline mustard merely inhibited tumor growth by 49%, melphalan caused tumor regression to 59% (six rats).

The growth curves of individual tumors are presented in detail in figures 2 and 3 for the controls and the groups treated with ovariectomy or ovariectomy plus either aniline mustard or melphalan. Tumor masses < 0.06 g (average diameter < 0.05 cm) are not presented except for completely regressing tumors. Only when a recurring tumor reached a mass > 0.06 g has its regrowth been depicted.

Tumor Numbers

The multiple tumors induced by DMBA in Sprague-Dawley rats are mixed hormone-responsive and hormone-unresponsive growths (23,24). Ovariectomy reduced the total number of tumors observed. For the three groups of ovariectomized rats, the cumulative number of tumors appearing until the end of the observation period, or until death, averaged 21.3 per group compared to 30.7 per group for the three groups of intact rats. When the hosts that died from drug toxicity are disregarded, these numbers were 20.3 and 29.3, respectively. In the cytostatically treated groups, the total numbers of tumors per group did not differ significantly from those in the appropriate control groups.

Enzyme Levels

Levels of β-glucuronidase activity were determined in parallel groups of mammary tumors that were induced similarly and selected strictly according to the same criteria as for the chemotherapy experiment. Table 2 lists enzyme levels for limited numbers of tumors excised at various times after ovariectomy. Regression was histologically evident in the specimens from Day 7 on. In the mouse plasma cell tumor ADJ/PC6,[4] which is known to be highly sensitive to aniline mustard, β-glucuronidase levels were also determined. In a series of 13 tumors transplanted sc in female BALB/c mice, $17.9 \pm 4.2 \times 10^{-3}$ units/mg of protein were found.

DISCUSSION

The aim of this study has been to examine the possibility of a therapeutic synergism between ovariectomy and aniline mustard, based on enzyme changes in hormone-responsive tumors during hormone-induced regression. According to the data in table 1, 38% of the tumors regressed completely in response to therapeutic ovariectomy, 16% did not respond, and 46% regressed partially. Following the combined treatment with ovariectomy plus aniline mustard, there were no nonresponding tumors; 72% of the tumors regressed completely and 28% regressed partially. After the combination treatment, the mean minimal total tumor mass per rat was 2% of the tumor mass at Day 0. After ovariectomy alone, the minimal tumor mass averaged 25% of the Day 0 value. Aniline mustard alone was virtually ineffective but appeared synergistic in combination with ovariectomy.

Melphalan as a single agent was approximately as effective as ovariectomy, but the effect of combining ovariectomy with melphalan was not supe-

[4]Provided by Dr. K. R. Harrap, Chester Beatty Institute for Cancer Research, London, England.

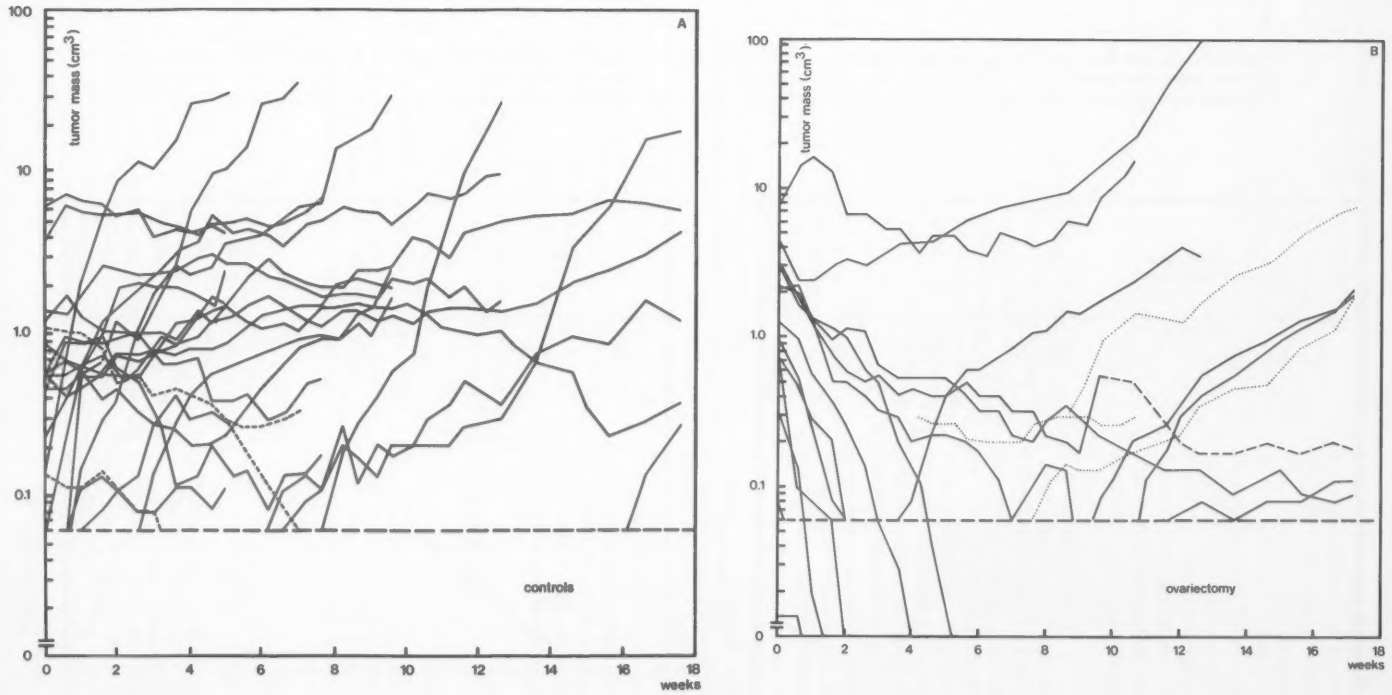

FIGURE 2.—Plots of individual tumor masses of DMBA-induced mammary tumors in Sprague-Dawley rats either untreated (A) or ovariectomized at Day 0 (B). ..... = Tumors arising after ovariectomy, --- = tumor regression that was apparently due to competition by a rapidly increasing tumor elsewhere in the same host. Volume reduction of partially regressing tumors is presented down to a limit of 0.06 g.

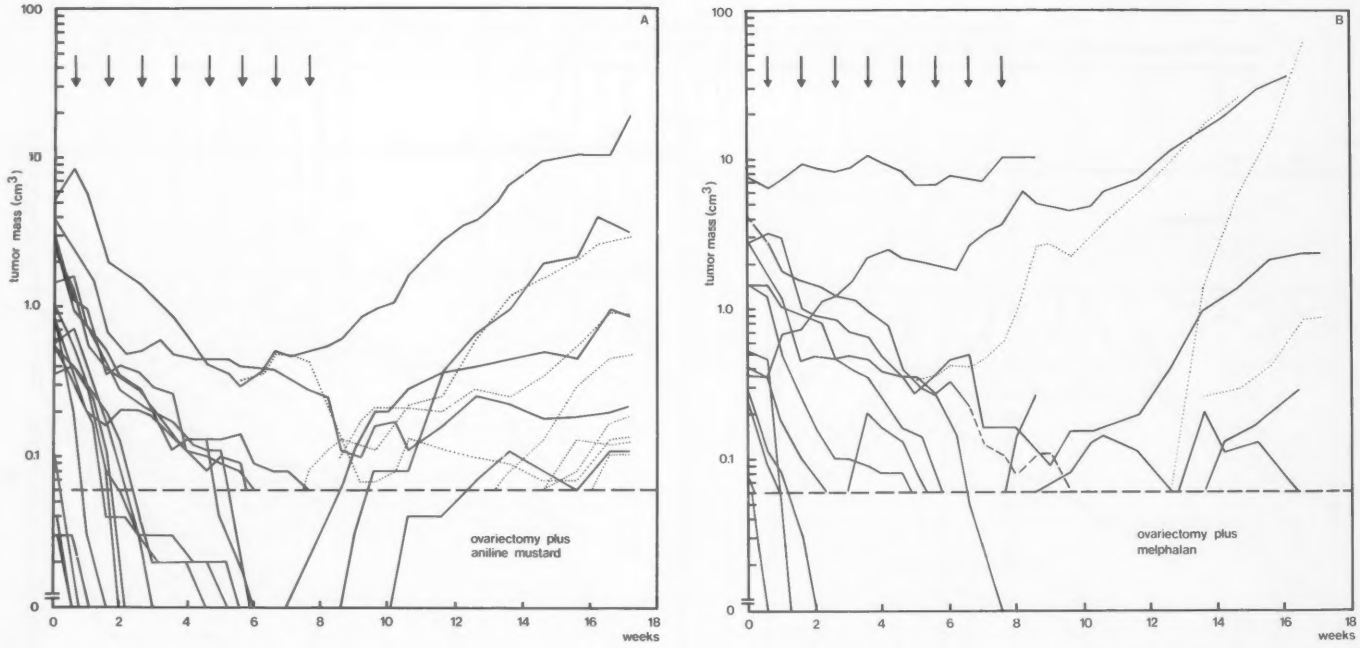

FIGURE 3.—Plots of individual tumor masses of DMBA-induced mammary tumors in Sprague-Dawley rats treated with aniline mustard (A) or melphalan (B). Treatment was started on Day 4, after ovariectomy at Day 0. ..... = Tumors arising after ovariectomy, --- = tumor regression that was apparently due to competition by a rapidly increasing tumor elsewhere in the same host. Volume reduction of partially regressing tumors is presented down to a limit of 0.06 g.

TABLE 2.—β-Glucuronidase levels in DMBA-induced mammary tumors at various times after ovariectomy

No. of days after ovariectomy	Intratumoral level of β-glucuronidase (units/mg of protein × 10^{-3})*
0	9.3 ± 5.1
4	17.8 ± 4.5
7	12.0 ± 4.2
14	8.1 ± 4.3
28	7.0 ± 2.7

*Values = mean ± SD.

rior to that of either treatment alone (table 1). The effect of ovariectomy plus melphalan (10 mg/kg/week given orally for 8 weeks in this study) was roughly comparable to that described by Griswold et al (25) for treatment with 2-α-methyldihydrotestosterone proprionate combined with melphalan (6 mg/kg/week given ip for 3 weeks): 65% and 50% of the animals, respectively, obtained a ≥ 50% reduction in total tumor mass. An explanation for the relatively poor results with melphalan combined with ovariectomy may be found in the cell cycle specificity of the alkylating drugs (26). The ^{3}H-thymidine labeling index has been found to decrease sharply in DMBA-induced rat mammary tumors during regression after ovariectomy (27), indicating a lowering of the growth fraction.

On the weekly schedule that has been applied for both cytotoxic drugs, aniline mustard was less effective than melphalan in intact animals; in the combination treatments, aniline mustard appeared superior. The comparison between both drugs is provisional, however. It is based upon a tentative conclusion (*see* Results) about equitoxicity of the applied doses in normal rats. Although the mortality data in the experimental groups are corroborative, the equivalence of the drug doses cannot be considered proven. For the moment the appropriate conclusion from the therapeutic results appears to be that aniline mustard might be less effective as a single agent than melphalan but might become a more effective drug than melphalan when combined with ovariectomy.

A high intratumoral level of the enzyme β-glucuronidase, which catalyzes deconjugation of the end product of aniline mustard metabolism (*p*-hydroxyaniline mustard *O*-glucuronide), is assumed to be responsible for the marked activity of aniline mustard against a few transplantable mouse tumors (2,6–8). Increased levels of several lysosomal enzymes have been reported in hormone-responsive mammary tumors in response to endocrine ablation (10–14). The data in table 2 confirm that increased β-glucuronidase levels occurred shortly after ovariectomy. A ratio of 1.9 was found for the enzyme level at Day 4 compared to Day 0; the levels were significantly different according to Student's t test ($P = 0.0133$). The ratio and the time course of enzyme activities in the present series of tumors are comparable to those that have been reported in individual DMBA-induced tumors sampled sequentially during regression (14). The β-glucuronidase activities observed 4 days after ovariectomy were similar to those in the aniline mustard-sensitive mouse plasma cell tumor ADJ/PC6 that was studied for comparison ($17.8 \pm 4.5 \times 10^{-3}$ and $17.9 \pm 4.2 \times 10^{-3}$ units/mg of protein, respectively).

An important point not covered by the present data is whether the higher level of β-glucuronidase occurring during regression in a hormone-responsive tumor might be more readily accessible to the drug conjugate *p*-hydroxyaniline mustard *O*-glucuronide than in a growing tumor. An indication that this may be so is found in an increased ratio of free over particulate-bound enzyme for at least 7 days following endocrine ablation, as reported by Lanzerotti and Gullino (11). In view of newer knowledge concerning the occurrence of nonlysosomal enzyme in the endoplasmic reticulum (28) and Golgi apparatus (29) of hormone-stimulated mouse kidney, more detailed information concerning the intracellular distribution of β-glucuronidase in mammary tumors seems required before any estimate of the availability of the enzyme for glucuronide deconjugation in regressing tumors can be made. An altered distribution might enhance the potential benefit that increased quantities of enzyme (11) appear to have for intratumoral activation of the cytostatically inactive *O*-glucuronide of *p*-hydroxyaniline mustard.

REFERENCES

1. Ross WCJ. Aryl-2-halogenoalkylamines. Part I. J Chem Soc:183, 1949.
2. Connors TA, Farmer PB, Foster AB, et al. Metabolism of aniline mustard [*N*,*N*-di-(2-chloroethyl)aniline]. Biochem Pharmacol 22:1971–1980, 1973.
3. Whisson ME, and Connors TA. Cure of mice bearing advanced plasma cell tumours with aniline mustard. Nature 206:689–691, 1965.
4. Artico M, and Ross WCJ. Aryl-2-halogenoalkylamines-XXIV. Derivatives of *o*-, *m*- and *p*-aminophenol: synthesis and antineoplastic activities. Biochem Pharmacol 17:893–898, 1968.
5. Bukhari MA, Everett JL, and Ross WCJ. Aryl-2-halogenoalkyl-amines-XXVI. Glucuronic, sulphuric and phosphoric esters of *p*-di-(2-chloroethyl)aminophenol. Biochem Pharmacol 21:963–967, 1972.
6. Ball CR, and Double JA. Enzyme activated anti-tumour agents. Conjugates of *p*-hydroxyaniline mustard as substrates for hydrolytic enzymes. Biochem Pharmacol 23:3173–3177, 1974.
7. Connors TA, and Whisson ME. Cure of mice bearing ad-

vanced plasma cell tumours with aniline mustard: the relationship between glucuronidase activity and tumour sensitivity. Nature 210:866–867, 1966.

8. Double JA, and Workman P. A new high-glucuronidase mouse tumor curable by aniline mustard therapy. Cancer Treat Rep 61:909–911, 1977.
9. Young CW, Yagoda A, Bittar ES, et al. Therapeutic trial of aniline mustard in patients with advanced cancer. Cancer 38:1887–1895, 1976.
10. Shamberger RJ. Lysosomal enzyme changes in growing and regressing mammary tumours. Biochem J 111:375–383, 1969.
11. Lanzerotti RH, and Gullino PM. Activities and quantities of lysosomal enzymes during mammary tumor regression. Cancer Res 32:2679–2685, 1972.
12. Cutts JH. Enzyme activities in regressing estrone-induced mammary tumors of the rat. Cancer Res 33:1235–1237, 1973.
13. Nicholson RI, Bagnal I, and Davies M. Lysosomal enzymes in 7,12-dimethylbenz(α)anthracene-induced mammary carcinomas after ovariectomy. Eur J Cancer 9:313–318, 1973.
14. Nicholson RI. Influence of altered lysosomal enzyme activities on the regression of DMBA-induced rat mammary tumours. Eur J Cancer 13:1225–1230, 1977.
15. Greenbaum AL, Slater TF, and Wang DY. Lysosomal enzyme changes in enforced mammary gland involution. Biochem J 97:518–522, 1965.
16. Brandes D, Anton E, and Barnard S. Lysosomes and cellular regressive changes in rat mammary gland involution. Lab Invest 20:465–471, 1969.
17. Helminen HJ, and Ericsson JLE. Quantitation of lysosomal enzyme changes during enforced mammary gland involution. Exp Cell Res 60:419–426, 1970.
18. Teller MN, Stock CC, Merker PC, et al. Biologic characteristics and chemotherapy of 7,12-dimethylbenz-(α)anthracene-induced tumors in rats. Cancer Res 26:245–252, 1966.
19. Griswold DP, Skipper HE, Laster WR, Jr, et al. Induced mammary carcinoma in the female rat as a drug evaluation system. Cancer Res 26:2169–2180, 1966.
20. Mead JAR, Smith JN, and Williams RT. Studies in detoxication. The biosynthesis of the glucuronides of umbelliferone and 4-methylumbelliferone and their use in fluorimetric determination of β-glucuronidase. Biochem J 61:569–574, 1955.
21. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
22. Schmidt LH, Fradkin R, Sullivan R, et al. Comparative pharmacology of alkylating agents. Cancer Chemother Rep suppl 2, Parts I, II, III: 1–1528, 1965.
23. Huggins C, Briziarelli G, and Sutton H. Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumors. J Exp Med 109:25–42, 1959.
24. Griswold DP, Jr, and Green CH. Observations on the hormone sensitivity of 7,12-dimethylbenz(α)anthracene-induced mammary tumors in the Sprague-Dawley rat. Cancer Res 30:819–826, 1970.
25. Griswold DP, Jr, Dykes DJ, Kelley CA, et al. Approaches to combination chemotherapy in rat, mouse, and hamster tumors. Cancer Chemother Rep Part 2, vol 4 (1):99–108, 1974.
26. Van Putten LM, Lelieveld P, and Kram-Idsenga LKJ. Cell-cycle specificity and therapeutic effectiveness of cytostatic agents. Cancer Chemother Rep 56:691–700, 1972.
27. Simpson-Herren L, and Griswold DP, Jr. Studies of the kinetics of growth and regression of 7,12-dimethylbenz(α)anthracene-induced mammary adenocarcinoma in Sprague-Dawley rats. Cancer Res 30:813–818, 1970.
28. Swank RT, and Paigen K. Biochemical and genetic evidence for a macromolecular β-glucuronidase complex in microsomal membranes. J Mol Biol 77:371–389, 1973.
29. Marsh CA, Lin CW, and Fishman WH. Golgi β-glucuronidase of androgen-stimulated mouse kidney. Biochem J 142:491–497, 1974.

Efficacy of Piperazinedione Prior to Bone Marrow Transplantation: Studies in a Rat Model for Human Acute Myelocytic Leukemia[1,2]

Anton Hagenbeek* and Anton C. M. Martens[3]

SUMMARY

The efficacy and toxicity of piperazinedione were investigated in a Brown Norway rat model for acute myelocytic leukemia (BNML). Treatment was started at a stage which is comparable to a "full-blown" relapse in human patients. Early toxicity-induced deaths due to the very strong myelosuppressive effect of the drug could be prevented by bone marrow transplantation followed by repeated blood transfusions. However, with a total dose of 16 mg/kg (human dose, 85 mg/m^2), death caused by GI tract and lung damage was inevitable. As judged by prolongation of survival, at the most a 9-log cell-kill was achieved. No cures were obtained, since the total tumor load at the start of treatment was $> 10^9$ cells (about 5×10^9). The possible presence of drug-resistant cells was not excluded. No significant differences in efficacy were found between daily "push" treatment and split daily doses using total doses of 10–12 mg/kg.

[Cancer Treat Rep 65:575–582, 1981]

Piperazinedione, a fermentation product of *Streptomyces griseoluteus* (1), has been shown to be effective against a variety of animal and human tumors (2–5). The drug seems to act mainly as an alkylating agent (6). With other alkylating agents such as cyclophosphamide, BCNU, and melphalan, there is minimal or no cross-resistance as determined in the L1210 mouse leukemia model (7,8). The dose-limiting toxicity is hematologic (9). Severe bone marrow aplasia is rapidly induced. Therefore, the drug is not generally used in clinical cancer treatment.

However, from recent phase II studies by Dicke et al (10,11), piperazinedione appeared to be very effective in reducing the tumor load in patients with relapsed acute leukemia prior to autologous bone marrow transplantation. In all 21 patients, a total dose of 50 mg/m^2 followed by 750–950 cGy total-body irradiation cleared the leukemic cells from the bone marrow. Besides the hematologic toxicity, mild nausea and vomiting was observed. About one half of the patients achieved a complete remission. The median remission duration was 4 months (range, 2–14). This is a significant result, if the extremely poor prognosis of relapsed patients with acute leukemia (particularly acute myelocytic leukemia [AML]) treated with conventional chemotherapy is taken into account.

Since it is (*a*) rather difficult, if not impossible, in human patients to determine the degree of tumor load reduction, and (*b*) time consuming to prescribe the optimal treatment regimen in terms of drug doses and time intervals, studies were performed in a transplantable rat leukemia model (BNML), which shares many characteristics with human acute (pro-)myelocytic leukemia (12).

MATERIALS AND METHODS

Experimental Animals

The experiments were performed with the inbred Brown Norway (BN) rat strain produced in the

[1]Received Aug 14, 1979; revised Sept 12, 1980; accepted Sept 22, 1980.

[2]Supported by the Koningin Wilhelmina Fonds of the Dutch National Cancer League (grant No. RBI 76/4).

[3]Radiobiological Institute TNO, Rijswijk, The Netherlands.

**Reprint requests to*: Dr. A. Hagenbeek, Transplantation Biology Unit, UCLA School of Medicine, Center for Health Sciences, Los Angeles, CA 90024.

Rijswijk colony. Male rats between 15 and 20 weeks of age were used (body weight, 220–290 g).

Rat Leukemia Model

The rat leukemia model (BNML) has been described in detail elsewhere [origin, classification, transplantation procedure, growth characteristics, etc (12,13)]. The leukemia was induced in a female BN rat by 9,10-dimethyl-1,2-benzanthracene. It shows a reproducible growth pattern upon intravenous cellular transfer within the BN rat strain. Cytologically and cytochemically it is similar to human acute promyelocytic leukemia. Further analogies with the human disease are: (*a*) a slow growth rate (10^7 BNML cell kill after 18–23 days; growth fraction, 0.60–0.40); (*b*) a severe suppression of normal hematopoiesis due to an absolute numerical decrease in the number of normal hematopoietic stem cells; (*c*) diffuse intravascular coagulation; (*d*) prolonged blood transit time of leukemic cells (34–36 hours); (*e*) response to chemotherapy as in human AML; (*f*) presence of clonogenic leukemic cells (no in vitro colony formation; TD50, 25 cells); (*g*) low antigenicity; and (*h*) no evidence for a virus as an etiologic agent.

Piperazinedione

Piperazinedione, produced by the National Cancer Institute, Bethesda, MD, was obtained through the courtesy of Dr. K. A. Dicke (Department of Developmental Therapeutics, M. D. Anderson Hospital and Tumor Institute, Houston, TX). It was dissolved in sterile water and injected either ip or iv (tail vein) in a volume of about 0.5 ml. Treatment was always started on Day 13 or 14 after inoculation with 10^7 BNML cells. This stage of disease is equivalent to the phase of "full-blown" relapse in human AML. The approximate number of tumor cells present is 5×10^9 (*see* below). Each group consisted of six to 12 rats.

Supportive Care

Bone marrow transplantation.—Within 24 hours after the last injection of piperazinedione, 6×10^7–10^8 isologous normal bone marrow cells were injected into a tail vein. This cell dose is more than enough to restore normal hematopoiesis and thus prevents the rats from dying of drug-induced bone marrow aplasia (14).

Transfusion of blood cells.—In some experiments, 1.5 ml of packed erythrocytes, leukocytes, and platelets were injected once a day for 4 consecutive days during the interval between treatment and the stage at which a sufficient number of mature end cells are produced by the transplanted bone marrow. The rapidly induced aplasia could thus be temporarily overcome. Blood was obtained from normal BN rats through puncture of the abdominal aorta under ether anesthesia.

Parameters of Evaluation

Hematologic followup.—In the first experiment, the total number of peripheral leukocytes and platelets and the percentage of reticulocytes were determined at different times after treatment in blood samples taken from tail veins. This method has been previously described (15).

Quantitative determination of tumor load reduction.—As previously reported (12), the tumor load on Day 13 or 14 is about 5×10^9. This number was derived from increments in organ weights (1 g = 10^9 cells), cell counts in organ suspensions (eg, femoral bone marrow), and bioassays. The log cell-kill induced by the various treatment regimens was derived from differences in median survival times between treated and nontreated rats, taking into account the duration of treatment (days). Given the linear relationship between the number of inoculated leukemic cells and the survival time, it can be deduced that 1-log cell-kill corresponds to an increase in lifespan of 4 days (16). Obviously, this requires that the repopulation kinetics of residual leukemic cells in treated rats are similar to the kinetics of proliferation of nontreated controls. Previous studies have indicated that this is the case for tumor loads ranging from 10^2 to 10^7 cells (13).

RESULTS

M. D. Anderson Protocol

At the M. D. Anderson Hospital and Tumor Institute, the original conditioning protocol for patients with acute leukemia prior to bone marrow transplantation included, besides total-body irradiation, two iv injections of 25 mg of piperazinedione/m^2, with a 24-hour interval between injections (10,11). According to Freireich et al (17) this corresponds to single doses of 4.73 mg/kg for the rat. Therefore, in the first experiment, doses of 5 mg/kg ip were used. Hematologic parameters are presented in figure 1. The number of leukocytes and platelets show a severe decrease shortly after treatment (fig 1, A and B). This suppression lasts until about Day 23, ie, 10 days after the start of treatment or 8 days after bone marrow transplantation. The percent of reticulocytes does not differ between treated rats and nontreated controls. After Day 23,

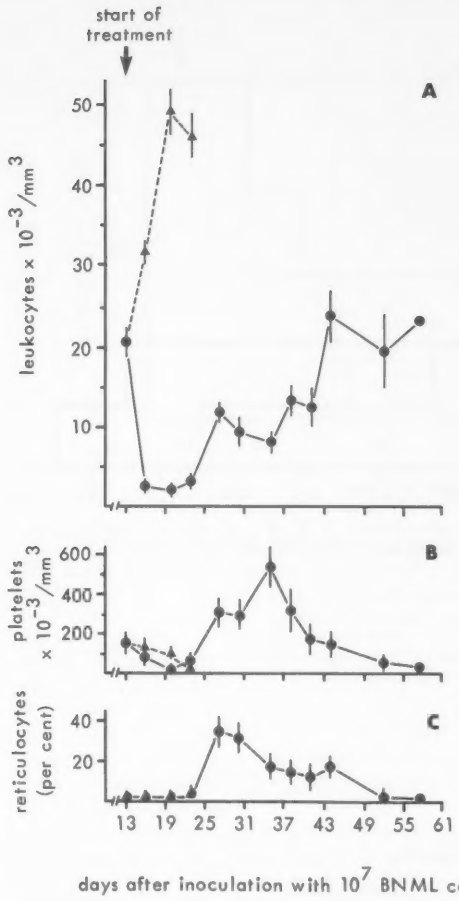

FIGURE 1.—Hematologic followup after 5 mg of piperazinedione/kg ip every 24 hrs × 2. 6 × 10^7 normal isologous bone marrow cells were transplanted 24 hrs after 2nd injection of piperazinedione. Each point = mean ± SE. ● ——— ● = 6 treated rats; ▲ ------- ▲ = 6 nontreated controls. Curves: A = leukocytes; B = platelets; C = reticulocytes.

platelet and reticulocyte numbers show an overshoot and later a decrease, again due to outgrowth of residual leukemia. Leukocytes increase steadily to about 20 × 10^6/ml in the terminal stage. At that time 70% are classified as leukemic cells.

In figure 2, the survival times are plotted. Note that a second treatment group is included, ie, 5 mg of piperazinedione/kg ip every 24 hours × 2 without subsequent bone marrow transplantation. In both treatment groups 50%–67% toxicity-induced early deaths were observed. These were mainly due to severe aplasia (hemorrhages through thrombocytopenia; lung infections through leukopenia). In the group which did not receive bone marrow, a slightly higher incidence of early death was observed. The survivors showed a tendency to die a little earlier than survivors in the bone marrow transplantation group. However, because of the relatively small numbers of animals no definite conclusions can be drawn so far. In later experiments, the early deaths could be overcome by administering fresh blood transfusions during the first days after piperazinedione treatment and bone marrow transplantation. With regard to the bone marrow transplantation group, the difference between the median survival time (MST) of the remaining treated rats and the nontreated controls was 30 days (55 − 23 days corrected for the duration of treatment, ie, 2 days). This corresponds to a 7.5-log cell-kill. Finally, all long-term survivors died from leukemia.

The double dose (9.46 mg/kg ip) was not tolerated; all rats died from drug toxicity.

It should also be mentioned that ip and iv administration of piperazinedione yielded similar results.

Split Daily Doses Versus Daily Push Treatment

In view of the unacceptable toxicity-induced deaths with the 5-mg/kg regimen, the total daily dose was decreased to 4 mg of piperazinedione/kg. Furthermore, in these series of experiments split doses (2 × 2 mg/kg/day at an 8-hour interval) were compared with push treatment (4 mg/kg/day). This was done to see whether: (*a*) toxic effects could be reduced; and (*b*) any synchronization of leukemic cells traversing the cell cycle and a possible phase-specific action of piperazinedione could be detected. Treatment was given for 2, 3, or 4 days.

Split Daily Doses

Figure 3 shows the survival times after 2 × 2 mg/kg ip every 24 hours × 2. There were no toxicity-induced deaths. The prolongation of MST was 24 days as compared with nontreated controls. This means that this treatment regimen induced a 6-log cell-kill.

In the next experiment, 1 extra day of treatment was added (2 × 2 mg/kg ip every 24 hours × 3). One group of treated rats received packed cells on each of Days 18–21. Almost 60% of early deaths due to severe aplasia were observed in the treated group (A) which did not receive packed cells (fig 4). If blood transfusions were given, no toxicity occurred (group B). Beyond Day 40, all rats died from leu-

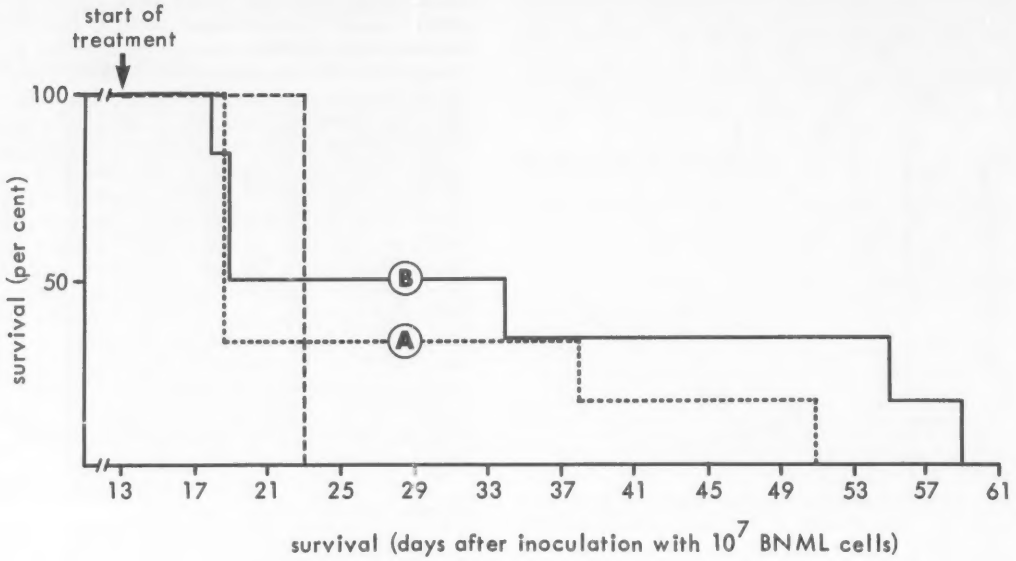

FIGURE 2.—Survival after 5 mg of piperazinedione/kg ip every 24 hrs × 2. -------- = group A: 6 treated rats, no subsequent bone marrow transplantation; ———— = group B: 6 treated rats, 6×10^7 normal isologous bone marrow cells were transplanted 24 hrs after 2nd injection of piperazinedione; ------ = 6 nontreated controls.

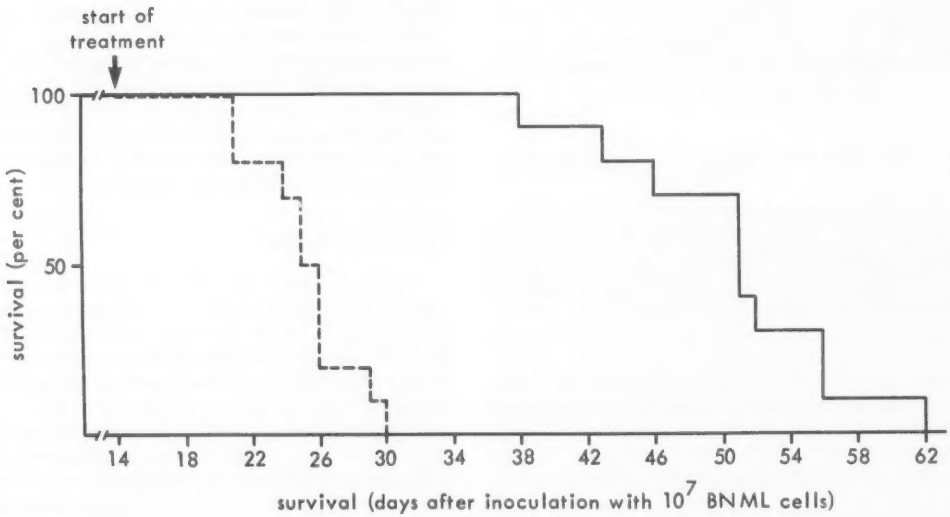

FIGURE 3.—Survival after 2 × 2 mg of piperazinedione/kg ip every 24 hrs × 2. 10^8 normal isologous bone marrow cells were transplanted 24 hrs after last injection of piperazinedione. ———— = 10 treated rats; -------- = 10 nontreated controls.

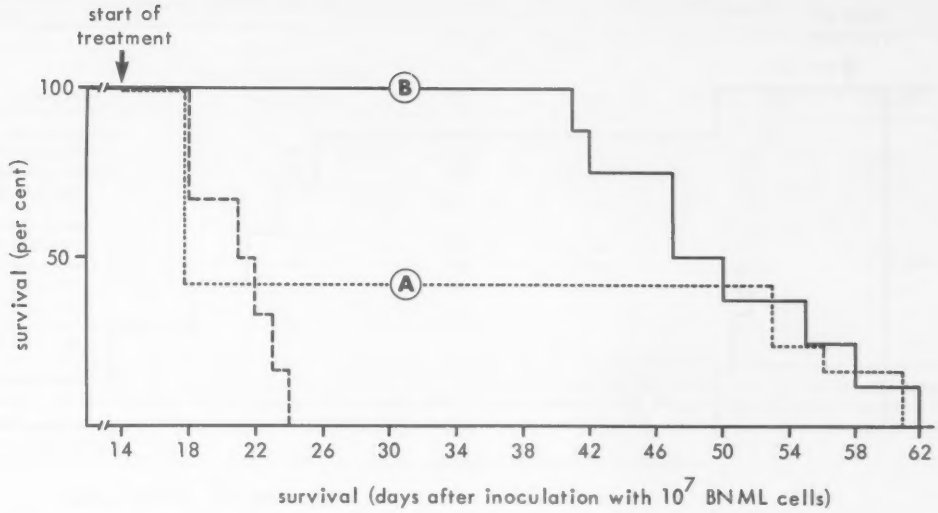

FIGURE 4.—Survival after 2 × 2 mg of piperazinedione/kg ip every 24 hrs × 3. 10^8 normal isologous bone marrow cells were transplanted 24 hrs after last injection of piperazinedione. ------- = group A: 12 treated rats; ———— = group B: 8 treated rats, 1.5-ml packed cells iv on each of Days 18–21; -------- = 6 nontreated controls.

kemia. Prolongation of MST was 32 days for the remaining rats in group A, and 26 days for group B. There is quite some spread in survival times, particularly in group B. The survival times of the longest survivors show a complete overlap with those of the remaining rats in group A. In these rats a 9-log cell-kill is deduced.

Despite additional supportive care with packed cells, a 4-day course of treatment (2 × 2 mg/kg ip every 24 hours × 4) was not tolerated. All ten rats died within 1 week after treatment was started. Besides severe aplasia, extensive hemorrhages in the gastrointestinal tract and in the lungs were observed at autopsy.

Daily Push Treatment

With 4 mg/kg iv every 24 hours push × 3 followed by bone marrow transplantation and repeated infusions with packed cells, only one of seven rats died from toxicity (fig 5). The prolongation of MST of the remaining rats was 27 days. This corresponds with a 7-log cell-kill.

Finally, the efficacies and concomitant toxic effects of the different regimens applied are summarized in table 1. Also note that the ranges of lifespan prolongation are indicated.

DISCUSSION

The data reported indicate a strong myelosuppressive and antileukemic activity of piperazinedione. Additional supportive care, ie, repeated transfusions of packed erythrocytes, platelets, and leukocytes, prevented early death due to rapidly induced severe aplasia (fig 4: compare curves A and B). If these prerequisites are taken care of, toxicity-induced deaths do not occur with, in these experiments, a maximal total dose of 12 mg of piperazinedione/kg of body weight followed by isologous bone marrow transplantation. When this dose was increased to 16 mg/kg (human dose, 85 mg/m²), all rats died from irreversible toxicity (GI tract, lungs [table 1]). Folk et al (9) also reported on degeneration of GI mucosa, pneumonia, and pulmonary edema in dogs and monkeys treated with piperazinedione. The sensitivity of the rat to piperazinedione seems to differ from that of man. The Houston protocol presently used includes a total dose of 75 mg/m². Preliminary data indicate that in man no comparable toxicity is observed (K. A. Dicke, personal communication).

With regard to the efficacy of the various treatment regimens applied, the following conclusions are drawn. First, there was a considerable spread of survival times in the various treatment groups

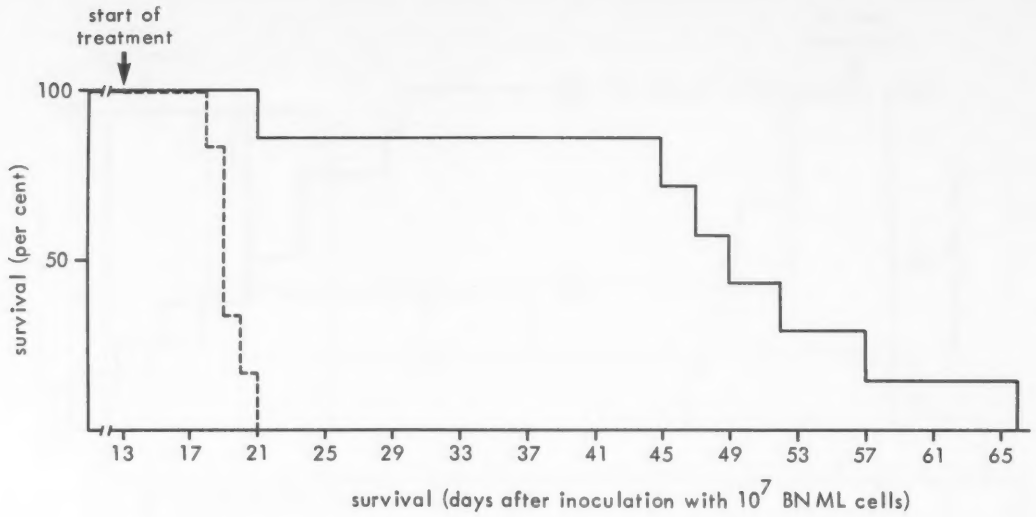

FIGURE 5.—Survival after 4 mg of piperazinedione/kg iv eve y 24 hrs × 3. 10^8 normal isologous bone marrow cells were transplanted 24 hrs after last injection of piperazinedione. ———— = 7 treated rats, 1.5-ml packed cells iv on each of Days 17–20; -------- = 6 nontreated controls.

(table 1). Therefore, the deduction of decrease in tumor load, expressed in log cell-kill and based on prolonged median survival time, is not completely valid. Although the earliest dying animals all showed signs of overt leukemia, a poorer general condition might have been responsible for their relatively early deaths, as compared with "stronger" and thus longer surviving rats in the same treatment group. It seems unlikely that within one experimental group tumor load reduction would vary between 2 and 9 decades (table 1). However, host variation in drug absorption, metabolism, and excretion, even with inbred animals, might have contributed to the differences observed. From the ranges of prolonged survival (table 1), it is clear that there is almost a complete overlap of all regimens. Therefore, split daily doses do not seem to be preferable above daily push treatment. Piperazinedione probably does not act as a cycle- or phase-specific drug. Studies with pulse cytophotometry did not show any perturbations of the distribution of leukemic cells over the cell cycle after piperazinedione treatment (A. Hagenbeek and A. Martens, unpublished observations). Therefore, the proposed alkylating activity of this drug (6) is supported by these studies in the BNML.

The original Houston protocol (5 mg/kg every 24 hours × 2 in the rat) is certainly not significantly less effective than the various other regimens applied if one looks at the longest survivors. Increasing the total dose to 12 mg yielded some rats with a prolonged survival time of 38–44 days (table 1). One-log cell-kill corresponds to an increase in lifespan of 4 days. An increase in lifespan of 38–44 days, ie, 45–50 days after the end of treatment, would thus correspond to a 10–11-log cell-kill (ie, 10–100 g of tumor tissue). Obviously, this is not realistic. At the time of treatment about 5×10^9 leukemic cells are present. In the longest surviving rats a ≥ 9-log cell-kill has been achieved. Only a few viable leukemic cells are left. Theoretically, piperazinedione might have induced, eg, a 10-log cell-kill. In that situation, the surviving leukemic cells might represent a resistant clone. At present, there are no data to prove or to disprove this possible mechanism. In previous experiments where low numbers of leukemic cells were inoculated into normal recipient rats, it was found that a survival time of 45–50 days corresponded to an original inoculum of 10–100 cells (16). This number of residual leukemic cells is thought to be present after treatment in those rats that survive about 50 days (figs 4 and 5) from the end of treatment. Their kinetics of regrowth apparently differ from those of larger numbers of surviving tumor cells. Only for inocula of 10^2–10^7 leukemic cells does the rule "4 days prolonged survival with every tenfold decrease in the number of

TABLE 1.—Efficacy and toxicity of piperazinedione treatment in the BNML model*

Regimen	Early toxicity-induced deaths (%)	Increase in lifespan (days)†		Log cell-kill	
		MST	Range	Based on MST	Range
A. Daily push treatment					
5 mg/kg every 24 hrs × 2	50	30	9–34	7.5	2–8.5
4 mg/kg every 24 hrs × 3 + packed cells	15	27	23–44	7	6–≥ 9
B. Split daily doses					
2 × 2 mg/kg every 24 hrs × 2	0	24	11–35	6	3–9
2 × 2 mg/kg every 24 hrs × 3	60	32	29–37	8	7–≥ 9
2 × 2 mg/kg every 24 hrs × 3 + packed cells	0	26	17–38	6.5	4–≥ 9
2 × 2 mg/kg every 24 hrs × 4 + packed cells	100	—	—	?	—

*Treatment was started on Day 13 or 14 after inoculation with 10^7 leukemic cells. All rats received 6 × 10^7–10^8 normal isologous bone marrow cells 24 hrs after the last injection of piperazinedione. Interval between 2 injections of piperazinedione/day was 8 hrs. Packed cells: 1.5 ml of packed cells were infused daily for 4 consecutive days, starting 24 hrs after bone marrow transplantation. Each group consisted of 6–12 rats.

†As determined by: (MST-treated rats) – (MST nontreated controls) – (duration of treatment).

injected leukemic cells" apply. Smaller numbers of surviving tumor cells are supposed to traverse a certain lag phase during which expected exponential growth does not take place (13). This period might be comparable to the stage of quiescence which seems to occur in certain patients with acute leukemia who have a long-lasting remission after chemotherapy. Whether the last remaining leukemic cells survived in these experiments because (*a*) the total dose of piperazinedione was too low (first-order kinetics in cell-killing), (*b*) they were insensitive to piperazinedione, or (*c*) they were hidden in sanctuaries which cannot be reached by the drug, remains to be investigated. Obviously, if they could be sterilized, cure would be obtained. Indeed, preliminary data indicate that combination of piperazinedione with total-body irradiation can cure about 40% of the rats (A. Hagenbeek and A. Martens, unpublished observations).

If these data may be extrapolated to man, it seems that piperazinedione alone will not completely eradicate all leukemic cells in patients with acute leukemia at relapse. They are thought to bear at least > 10^{10} leukemic cells, whereas piperazinedione induces maximally a 9-log cell-kill. Even if a ≥ 10-log cell-kill occurred, the survival of drug-resistant leukemic cells might be a major obstacle to curative treatment. Hopefully, forthcoming clinical data will finally confirm the findings suggested by this experimental work. Above all, it should be remembered that one rat leukemia model, although representative of the human disease, represents only one patient with leukemia.

REFERENCES

1. GITTERMAN CO, RICKES EL, WOLF DE, ET AL. The human tumor-egg host system. IV Discovery of a new antitumor agent: compound 593A. J Antibiot 23:305, 1970.
2. TARNOWSKI GS, SCHMID FA, HUTCHISON DJ, ET AL. Chemotherapeutic effects of compound 593A (NSC-135758) on mouse leukemias and some transplanted animal tumors. Cancer Chemother Rep 57:21, 1973.
3. GOTTLIEB JA, FREIREICH EJ, BODEY GP, ET AL. Preliminary clinical evaluation of piperazinedione (P): a new crystalline antibiotic. Proc Am Assoc Cancer Res and ASCO 16:86, 1975.
4. JONES S, SALMON S, and SOUTHWEST ONCOLOGY GROUP. Phase II trial of piperazinedione (crystalline antibiotic) in lymphoma and multiple myeloma. Proc Am Assoc Cancer Res and ASCO 17:45, 1976.
5. JONES SE, TUCKER WG, HAUT A, ET AL. Phase II trial of piperazinedione in Hodgkin's disease, non-Hodgkin's lymphoma, and multiple myeloma: a Southwest Oncology Group study. Cancer Treat Rep 61:1617, 1977.
6. BROCKMANN RW, SHADDIX SC, ADAMSON DJ, ET AL. Inhibition of growth and of DNA synthesis in Adenocarcinoma 755 cells in culture by 3,6-bis(5-chloro-2-piperidinyl)-2-5-piperazinedione (NSC-135758) and evidence for its alkylating activity. Proc Am Assoc Cancer Res and ASCO 16:35, 1975.
7. SCHABEL FM, JR, TRADER MW, LASTER WR, ET AL. Studies with 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidinyl)-dihydrochloride. III. Biochemical and therapeutic effects in L1210 leukemias sensitive and resistant to alkylating agents: comparison with melphalan, cyclophosphamide and BCNU. Cancer Treat Rep 60:1325, 1976.
8. SCHABEL. FM, TRADER MW, LASTER WR, ET AL. Patterns of resistance and therapeutic synergism among alkylating agents. *In* Fundamentals in Cancer Chemotherapy (Karger, Basel, Switzerland). Antibiot Chemother 23:200, 1978.
9. FOLK RM, PETERS AC, PAVKOV KL, ET AL. Preclinical toxicological evaluation of 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl),-dihydrochloride (NSC-135758) in dogs and monkeys. Cancer Chemother Rep 5:37, 1974.

10. DICKE KA, MCCREDIE KB, SPITZER G, ET AL. Autologous bone marrow transplantation in patients with adult acute leukemia in relapse. Transplantation 26:169, 1978.
11. DICKE KA, SPITZER G, PETERS L, ET AL. Autologous bone marrow transplantation in relapsed adult acute leukaemia. Lancet 1:514, 1979.
12. HAGENBEEK A, and VAN BEKKUM DW, eds. *In* Proceedings of a Workshop on Comparative Evaluation of the L5222 and the BNML Rat Leukaemia Models and their Relevance for Human Acute Leukaemia. Leuk Res 1:75–255, 1977.
13. VAN BEKKUM DW, and HAGENBEEK A. Relevance of the BN leukemia as a model for human acute myeloid leukemia. Blood Cells 3:565, 1977.
14. VAN BEKKUM DW. The appearance of the multipotential hemopoietic stem cell. *In* Experimental Hematology Today (Baum SJ and Ledney GD, eds). New York, NY, Springer-Verlag, 1977, p 3.
15. HAGENBEEK A. Extracorporeal Irradiation of the Blood in a Rat Leukaemia Model (Mongr). Rÿswÿk, The Netherlands, Radiobiological Institute TNO, 1977, p 40.
16. HAGENBEEK A. Introduction of the BN myelocytic leukemia. Leuk Res 1:85, 1977.
17. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother Rep 50:219, 1966.

Experimental Studies on the Radiation-Modifying Effect of Bleomycin in Malignant and Normal Mouse Tissue in Vivo[1,2]

Jeanne Molin, Poul Erik Søgaard, and Jens Overgaard[3,4,*]

SUMMARY

The interaction between bleomycin (BLM) and radiation was studied in a C3H mammary carcinoma and its surrounding normal skin. In the skin, single and fractionated doses of sequential treatment with BLM (25 mg/kg) 24 hours prior to radiation therapy did not influence the response to irradiation, whereas simultaneous treatment with BLM given 15 minutes before radiation therapy enhanced the reaction to irradiation by a factor of 1.2 or 1.4 following treatment with one fraction or five fractions, respectively. The tumor response to irradiation was not influenced by a single sequential treatment, but five daily fractions of radiation therapy following five daily dose fractions of BLM increased the radiation dose needed to control 50% of the tumors, probably because the tumors continued to grow during the BLM treatment. Simultaneous treatment enhanced the response to irradiation by a factor of 1.2 after both single-dose and fractionated therapy. Based on these data it was concluded that none of the combined treatment schedules were able to produce a better therapeutic effect than radiation therapy alone. Furthermore, mortality due to lung fibrosis in mice treated with BLM indicated the marked toxicity of the drug. This toxicity was most pronounced after fractionated treatment and when radiation therapy and BLM were given simultaneously.

[Cancer Treat Rep 65:583–589, 1981]

Bleomycin (BLM) is frequently suggested as a useful adjuvant to radiotherapy. This has its basis in the potential mechanism by which BLM may interact with the response to irradiation in tumor tissue. In principle, three major types of interaction could suggest advantage of a combined approach. Pre-irradiation treatments with BLM can debulk a tumor, which in turn could facilitate reoxygenation and thereby result in a smaller radiation dose being needed to control the remaining tumor. BLM is also known to block cells in the transition between G_2 and M (1,2) and could theoretically be advantageous by partly synchronizing tumor cells into this relatively radiosensitive stage of the cell cycle. Finally, a simultaneous treatment with BLM and radiation may increase the radiosensitivity of cells, primarily due to a decreased repair of sublethal radiation damage (3–8).

BLM is often used in clinical studies, a rationale for such therapy being primarily based on in vitro observations, but there is only limited experience with the effect of BLM in combination with radiation therapy in experimental animal tumors (5,8–12). An interaction between this drug and radiation has, however, been demonstrated in several normal tissues in experimental animals and in man (13–19). Further, the small number of in vivo tumors have

[1]Received May 30, 1980; revised Sept 17, 1980; accepted Sept 30, 1980.

[2]Supported by Danish Cancer Society grant 24/79 and Krista and Viggo Petersen's Foundation.

[3]The Institute of Cancer Research (sponsored by the Danish Cancer Society) and Department of Oncology and Radiation Therapy, Radiumstationen, Aarhus C, Denmark.

[4]We thank H. Lundbeck & Co, Copenhagen, for supplying the bleomycin.

*_Reprint requests to_: Jens Overgaard, MD, The Institute of Cancer Research, Radiumstationen, 44 Nörrebrogade, DK-8000 Aarhus C, Denmark.

generally been tested by growth delay or by in vivo and in vitro assays after single-dose irradiation (5, 8, 9,11), and there is even less information on how this combined treatment given in fractionated schedules may influence the clinically important end point of local tumor control.

The aim of the present study was therefore to explore the rationale for the use of BLM as an adjuvant to radiation therapy by investigating the effect of sequential or simultaneous BLM and radiation treatment in an experimental tumor and its surrounding normal tissue. The treatment was given with single-dose or fractionated therapy in an effort to obtain an improved therapeutic ratio with the combined treatment.

MATERIALS AND METHODS

Animal Tumor System

The experimental system has previously been described in detail (20,21). Male and female C3D2F1/Bom (C3H/Tif ♀ × DBA/2 ♂) mice, 10–12 weeks old, were challenged with a spontaneously arisen C3H/Tif mammary carcinoma. The tumor was propagated by serial transplantation. Experimental tumors were produced by inoculating 5–10 μl of tumor homogenate into the foot on the right hind limb of the animals.

Treatment

Treatment was performed on tumors with a volume of approximately 200 mm^3 as determined by the formula $D1 \times D2 \times D3 \times \frac{\pi}{6}$, where D represents three orthogonal diameters.

Irradiation.—Tumors were treated with graded doses of radiation to produce dose-response data. The treatment was given with a conventional clinical X-ray machine with a dose rate of approximately 1.9 Gy/minute (factors: 250 kV, 15 mA, 2 mm Al filtration, and 1.1 mm Cu half-value layer. The unanesthetized animals were placed in a Lucite jig with the tumor-bearing leg loosely fixed with tape, so that the blood flow to the foot was not impaired. Radiation was given to tumors in a water bath at room temperature and with approximately 0.5 cm of water between the X-ray source and the tumor, to secure the homogenicity of the radiation dose. The remaining part of the animal was shielded with 5-mm lead.

BLM.—The drug was supplied by H. Lundbeck & Co., Ltd. (Copenhagen, Denmark). Immediately before injection, it was diluted with sterile water to a concentration of 1.5 mg/ml. This solution was injected ip into nonanesthetized mice in a dose of 25 mg/kg at each treatment.

Treatment schedules.—The different single-dose and fractionated treatment schedules applied are given in table 1.

Evaluation of Results

The effect of BLM alone on tumor growth was evaluated by calculating the mean time to a fivefold increase in tumor volume in each treatment group.

The response of animals to combined treatment was measured by determining the radiation dose which would, on an average, be expected to control 50% of the treated tumors (TCD50) at 120 days. The response of the normal tissue was measured by determining the radiation dose required to achieve a full moist desquamation of the irradiated limb

TABLE 1.—Treatment schedules

Treatment	Day of treatment									
	1	2	3	4	5	6	7	8	9	10
Single-dose										
Radiation alone	R*									
Simultaneous BLM and radiation†	BLM + R									
Sequential BLM and radiation	BLM	R								
Fractionated										
Radiation alone	R	R	R	R	R					
Simultaneous BLM and radiation†	BLM + R	BLM + R	BLM + R	BLM + R	BLM + R					
Sequential BLM and radiation	BLM	BLM	BLM	BLM	BLM	R	R	R	R	R

*R = radiation.
†BLM 15 min prior to radiation.

within 30 days in 50% of the animals (DD50). The TCD50 and DD50 values were computed by a logit analysis (22).

The effect of an additional BLM treatment on the response to radiation was evaluated by calculating the "enhancement ratio." This is the radiation dose required to obtain a given end point (TCD50 or DD50) with radiation therapy alone, relative to the radiation dose needed to obtain the same response with combined BLM and radiation treatment.

Additionally, a "therapeutic gain factor" (TGF) was calculated as the enhancement ratio for the tumor relative to the enhancement ratio for the normal tissue.

RESULTS

BLM Alone

The influence of BLM alone was studied by measuring the growth delay in the tumor. Figure 1 shows the growth curves after a single-dose or a fractionated course of BLM given with 25 mg/kg in each fraction. Although the tumor is not of the epidermoid type, BLM caused a significant growth delay, which was more pronounced after a fractionated treatment than following single-dose treatment (table 2). After completion of the drug treatment, the tumors resumed a normal growth pattern corresponding to that in controls, with a doubling time of approximately 2.5 days. No changes in the skin overlying the tumor were observed.

Combined BLM and Radiation Treatment

Effect on skin.—Sequential treatment with BLM and radiation, given either in a single-dose or fractionated schedule, did not result in any modification of the response to irradiation (tables 3 and 4). In contrast, BLM applied 15 minutes before each irradiation fraction (simultaneous treatment) resulted in a marked enhancement of the skin response. A single, simultaneous treatment resulted in an enhancement ratio of approximately 1.2 due to a reduction in the DD50 dose from 27.2 Gy with radiation therapy alone to 23.2 Gy with the combined treatment (table 3). A greater effect was seen with the five-fraction schedule; an enhancement ratio of 1.38 was observed, corresponding to an overall dose reduction of 15.7 Gy (table 4). Thus, BLM appears to have a greater radiosensitizing effect in the skin in a fractionated schedule than after single-dose treatment.

Effect on tumor.—No difference was noted between a single dose of BLM given 24 hours prior to radiation therapy (sequential treatment) and ir-

TABLE 2.—Effect of single-dose and fractionated BLM treatment on growth of a C3H mammary carcinoma

Treatment	No. of animals	Mean time to fivefold tumor volume (days ± SD)	Increase in time to fivefold tumor volume (%)
Untreated controls	26	6.4 ± 1.2	—
Single dose of BLM (25 mg/kg ip)	14	8.2 ± 1.1	28
5 daily fractions of BLM (25 mg/kg ip)	19	9.8 ± 1.6	53

TABLE 3.—Interaction between single doses of BLM and irradiation

Treatment	Tumor response		Skin response		TGF
	TCD50 (Gy)	Enhancement ratio	DD50 (Gy)	Enhancement ratio	
Radiation alone	55.9 (53.2–58.6)*	—	27.2 (25.4–29.1)	—	—
Sequential BLM† and radiation	55.4 (44.9–60.2)	1.01 (0.95–1.07)	28.1 (25.6–30.7)	0.97 (0.80–1.16)	1.04
Simultaneous BLM† and radiation	46.1 (38.5–55.2)	1.21 (1.08–1.36)	23.2 (21.2–25.5)	1.17 (1.08–1.27)	1.03

*Nos. in parentheses = 95% confidence limits.
†Dose, 25 mg/kg.

TABLE 4.—Interaction between fractionated doses of BLM and irradiation

Treatment	Tumor response			Skin response			
	TCD50 (Gy)	Enhancement ratio	F_R*	DD50 (Gy)	Enhancement ratio	F_R	TGF
Radiation alone	61.0 (54.4–68.2)†	—	0.10	56.7 (50.4–63.8)	—	0.63	—
Sequential BLM‡ and radiation	70.4 (53.8–92.2)	0.87 (0.72–1.03)	0.26	56.8 (48.8–65.8)	1.0 (0.89–1.12)	0.63	0.87
Simultaneous BLM‡ and radiation	49.7 (44.0–56.1)	1.23 (1.10–1.37)	0.09	41.0 (35.3–47.7)	1.38 (1.22–1.57)	0.54	0.89

*Recovered dose $F_R = \frac{D_N - D_1}{N - 1} \Big/ \frac{D_N}{N}$.

†Nos. in parentheses = 95% confidence limits.

‡Dose, 25 mg/kg/fraction.

radiation alone (table 3). A sequential treatment regimen with BLM, given daily for 5 days followed by five daily fractions of irradiation, increased the TCD50 value from 61.0 to 70.4 Gy. Thus, fractionated sequential treatment was less effective in the tumor than radiation therapy alone.

Simultaneous treatment, on the other hand, resulted in a reduced TCD50 dose of approximately 10 Gy after both single-dose or fractionated treatment (corresponding to enhancement ratios of 1.21 and 1.23, tables 3 and 4).

Therapeutic ratio.—An evaluation of the treat-

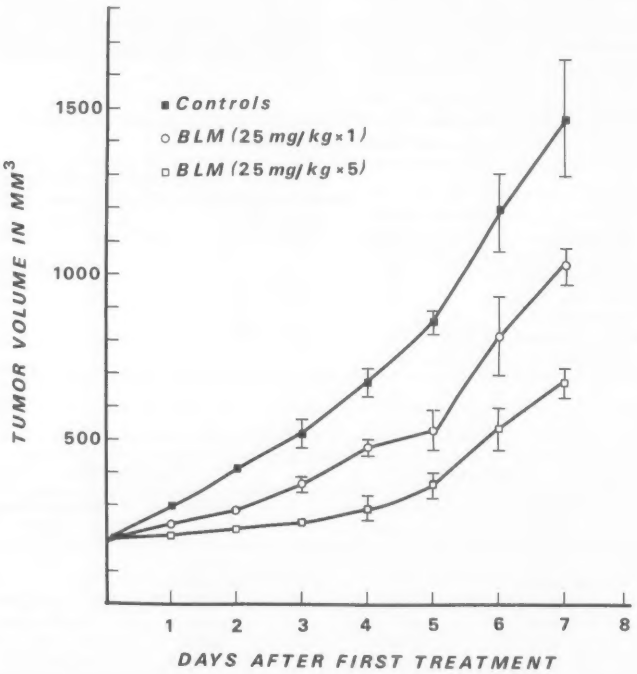

FIGURE 1.—Effect of a single dose or 5 daily treatments with BLM on the growth of a C3H mammary carcinoma. Vertical bars = SD.

TABLE 5.—Toxicity of BLM given in different combined schedules with irradiation

Treatment	Single-dose treatment		Fractionated treatment	
	No. of deaths/ No. of mice treated	Mortality rate (%)*	No. of deaths/ No. of mice treated	Mortality rate (%)*
Sequential BLM† and radiation	1/97	1.08	31/122	25.4
Simultaneous BLM† and radiation	12/103	11.6	113/200	56.2

*Differences are statistically significant ($P < 0.01$).
†Dose = 25 mg/kg/fraction.

ment response in the form of a TGF showed no significant improvement in any of the combined treatment schedules since BLM apparently affected the response to irradiation in the tumor to a lesser extent than in the normal tissue. The TGF values in both combined fractionated schedules were below 0.9, which may indicate a reduced therapeutic effect.

Toxicity

Within the 120-day observation period, no animal deaths were attributed to toxic effects of irradiation alone. On the other hand, BLM induced a profound toxicity which was most pronounced in the fractionated treatment schedules and was greater (to a statistically significant degree) following simultaneous treatment than after sequential application (table 5). Figure 2 shows the mortality and time to death of BLM-treated animals. Autopsy showed BLM-induced pulmonary fibrosis. Histopathologic examination of tissues from BLM-treated animals revealed no other organ involvement. Thus, mortality was judged to be a result of pulmonary dysfunction.

DISCUSSION

The present data indicate that BLM affects the response to ionizing radiation in an experimental tumor and its surrounding normal skin, depending on the treatment schedule applied.

In the skin, simultaneous treatment with BLM and radiation therapy caused a marked enhancement of the response to irradiation, with an enhancement ratio of about 1.2 following single-dose treatment. This observation is consistent with other experimental studies in different normal tissues (13,14,16–19). A further enhancement was observed if the simultaneous treatment was given in a fractionated schedule. This enhancement may be illustrated by consideration of the fraction of the dose recovered:

$$F_R = \frac{D_N - D_1}{N - 1} \Big/ \frac{D_N}{N}$$

where N is the number of fractions and D_1 and D_N are the doses necessary to achieve the same level of damage in 1 or N fractions, respectively. As seen in table 4, simultaneous BLM treatment caused a reduction in the F_R value from 0.65, observed with radiation therapy alone, to 0.54. Also, the average dose recovered between each fraction, expressed as $(D_N - D_1/N - 1)$ is included in the above formula. This recovered dose per fraction was for the skin response after irradiation alone (7.4 Gy), whereas the simultaneous treatment showed a recovered dose of 4.4 Gy. Thus, the simultaneous treatment caused a reduction in the repair of sublethal damage corresponding to approximately 3 Gy/fraction. This finding corresponds to the dose reduction of 4.0 Gy observed after single-dose treatment. Thus, the results are consistent with the assumption that BLM primarily interacts with radiation by reducing the repair of sublethal radiation damage. In contrast, a pretreatment with BLM for 1 or 5 days did not alter the response to irradiation in the skin. Consequently, it is unlikely that BLM induced any indirect effects, such as redistribution within the cell cycle, which could, theoretically, alter the response to irradiation in the present experimental system.

Regarding the tumor data, one might argue that the use of a mammary carcinoma is not a suitable model for testing the effect of BLM since this drug is known to be most effective against epidermoid carcinomas. However, the C3H mammary carcinoma is sensitive to BLM treatment, as evidenced by a dose-dependent growth delay, a finding which is in agreement with previous studies in a similar tumor system (23). The tumor response in our mammary carcinoma is more pronounced than the growth-delaying effect obtained by Juul Jørgensen (24) in

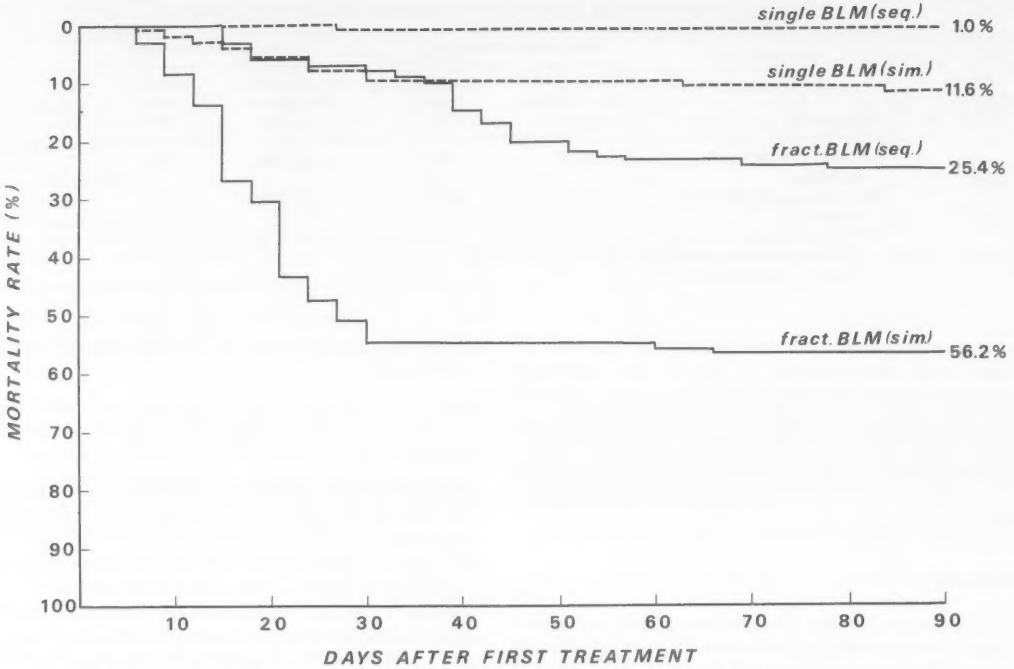

FIGURE 2.—Mortality and time of death due to toxicity in the different schedules of combined BLM and radiation therapy; seq = sequential, sim = simultaneous.

studies with an experimental squamous cell carcinoma. Therefore, the use of a mammary carcinoma does not seem to influence the validity of the clinical implications which may be drawn from our experiments.

In the tumor, a single simultaneous treatment with BLM reduced the TCD50 dose by 9.8 Gy. A TCD50 reduction of the same magnitude was observed with the fractionated treatment (11.3 Gy). The reason for absence of a fractionated effect on the enhancement ratio is probably complex. It cannot be explained by a reduced repair of sublethal damage alone since the F_R value was about 0.10 both with and without simultaneous BLM treatment (table 4). However, the response to irradiation after fractionated treatment in this tumor system is very much influenced by a rapid reoxygenation which may mask some of the mechanisms involved, especially because BLM apparently influences the response to irradiation in nutritionally deprived hypoxic cells only to a very small extent (25,26).

It may be surprising that 5 days of BLM treatment prior to five daily fractions of radiation therapy resulted in an increase in the TCD50 compared to the effect of irradiation alone. However, one must take into account that, although BLM causes a relative inhibition of the tumor growth, an increase in tumor volume did occur during the drug treatment (fig 1). Thus, the average tumor size at the time of radiation therapy in the sequentially treated group was 495 mm^3, compared to 195 mm^3 in the group treated with irradiation alone. If this increase in volume represents an increase in the overall number of clonogenic cells and if there is a constant fraction of hypoxic tumor cells, this would explain the increase in radiation dose necessary for tumor control (22).

None of the treatment schedules were designed with special attention to the potential BLM-induced synchronization of cells between G_2 and M. However, the continuous tumor growth observed with daily BLM treatment (fig 1) makes it unlikely that any significant synchronization occurs in the present tumor system.

Although some of the treatment schedules enhanced the tumor response to irradiation, all therapy schedules resulted in a similar or decreased therapeutic ratio. This was primarily because the

treatment induced a more pronounced effect on the normal tissue response than on the tumor response. A similar result has been observed in clinical studies in which BLM is given simultaneously with radiotherapy (27). It is therefore unlikely that BLM will have any major role as a radiosensitizing agent in clinical therapy (25). In tumors where BLM itself has a marked debulking effect (eg, penis cancer and some head and neck carcinomas), local control may be improved by a pre-irradiation drug treatment. However, to minimize irradiation side effects, such treatment should be given sequentially rather than simultaneously. This applies also to a situation where BLM is given with the intention of controlling systemic metastases rather than as an adjuvant to radiotherapy of the local tumor. It should be emphasized that any experimental evaluation of a potentially radiosensitizing agent must be performed in both tumor and critical normal tissue systems. Any conclusion drawn from a tumor response alone may not be valid to predict a potential clinical improvement since critical normal tissues may be included in the radiation field.

The significant increase in lung toxicity following simultaneous treatment compared to that following sequential therapy was surprising. It could be explained by scattered radiation to the thorax during the local tumor treatment, but there was no relationship between the given radiation dose and the lethality, and dose measurements in the thorax showed an almost complete protection from scattered radiation. Therefore, this problem is open for discussion.

REFERENCES

1. Barranco SC, and Humphrey RM. The effect of bleomycin on survival and cell progression in Chinese hamster cells in vitro. Cancer Res 31:1218–1223, 1971.
2. Kimler BF. The effect of bleomycin and irradiation on G_2 progression. Int J Radiat Oncol Biol Phys 5:1523–1526, 1979.
3. Bleehen NM, Gillies NE, Twentyman PR, et al. The effect of bleomycin and radiation in combination on bacteria and mammalian cells in culture. Br J Radiol 47:346–351, 1974.
4. Matsuzawa T, Onozawa M, Morita K, et al. Radiosensitization of bleomycin on lethal effect of mouse cancer cell in vitro. Strahlentherapie 144:614–617, 1972.
5. Sakamoto K, and Sakka M. The effect of bleomycin and its combined effect with radiation on murine squamous carcinoma treated in vivo. Br J Cancer 30:463–468, 1974.
6. Takabe Y, Miyamoto T, Watanabe M, et al. Synergism of X-rays and bleomycin on Ehrlich ascites tumour cells. Br J Cancer 36:391–395, 1977.
7. Terasima T, Takabe Y, and Yasukawa M. Combined effect of X-ray and bleomycin on cultured mammalian cells. Gan 66:701–703, 1975.
8. Wharam MD, Phillips TL, Kane L, et al. Response of a murine solid tumor to in vivo combined chemotherapy and irradiation. Radiology 109:451–455, 1973.
9. Begg AC, Kane LJ, Fu KK, et al. The effects of combined bleomycin and irradiation on a solid murine tumor. Int J Radiat Oncol Biol Phys 5:1503–1507, 1979.
10. Juul Jørgensen S. Time-dose relationships in combined bleomycin treatment and radiotherapy. Eur J Cancer 8:531–534, 1972.
11. Twentyman PR, Kallman RF, and Brown JM. The effect of time between X-irradiation and chemotherapy on the growth of three solid mouse tumors. V. Bleomycin. Int J Radiat Oncol Biol Phys 5:1499–1501, 1979.
12. Urano M, Ando K, Koike S, et al. Combined use of bleomycin in radiotherapy of a mouse mammary carcinoma. Gan 67:157–163, 1976.
13. Guigon M, Frindel E, and Tubiana M. Effects of the association of chemotherapy and radiotherapy on normal mouse skin. Int J Radiat Oncol Biol Phys 4:233–238, 1978.
14. Leith JT, Lewinsky BS, and Schilling WA. Modification of the response of mouse skin to X-irradiation by bleomycin treatment. Radiat Res 61:100–109, 1975.
15. Phillips TL, and Fu KK. Acute and late effects of multimodal therapy on normal tissues. Cancer 40:489–494, 1977.
16. ———. Quantification of combined radiation therapy and chemotherapy effects on critical normal tissues. Cancer 37:1186–1200, 1976.
17. ———. The interaction of drug and radiation effects on normal tissues. Int J Radiat Oncol Biol Phys 4:59–64, 1978.
18. Phillips TL, Ross GY, Goldstein LS, et al. The interaction of radiation and bleomycin in intestinal crypt cells. Int J Radiat Oncol Biol Phys 5:1509–1512, 1979.
19. Phillips TL, Wharam MD, and Margolis LW. Modification of radiation injury to normal tissues by chemotherapeutic agents. Cancer 35:1678–1684, 1975.
20. Overgaard J. Effect of misonidazole and hyperthermia on the radiosensitivity of a C3H mouse mammary carcinoma and its surrounding normal tissue. Br J Cancer 41:10–21, 1980.
21. ———. Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue in vivo. Int J Radiat Oncol Biol Phys 6:1507–1517, 1980.
22. Suit HD, Shalek RJ, and Wette R. Radiation response of C3H mouse mammary carcinoma evaluated in terms of cellular radiation sensitivity. *In* Cellular Radiation Biology. Baltimore, MD, Williams & Wilkins, 1965, pp 514–530.
23. Urano M, Fukuda N, and Koike S. The effect of bleomycin on survival and tumor growth in a C3H mouse mammary carcinoma. Cancer Res 33:2849–2855, 1973.
24. Juul Jørgensen S. Dose schedules in bleomycin treatment. Eur J Cancer 8:93–97, 1972.
25. Hall EJ. Bleomycin: drug summary. Int J Radiat Oncol Biol Phys 5:1537–1539, 1979.
26. Roizin-Towle L, and Hall EJ. Studies with bleomycin and misonidazole on aerated and hypoxic cells. Br J Cancer 37:254–260, 1978.
27. Muggia FM, Cortes-Funes H, and Wasserman TH. Radiotherapy and chemotherapy in combined clinical trials: problems and promise. Int J Radiat Oncol Biol Phys 4:161–171, 1978.

Synergistic Antileukemic Activity of Combinations of Two Nitrosoureas[1,2]

Glynn P. Wheeler,* Frank M. Schabel, Jr, and Mary W. Trader[3]

SUMMARY

A rationale based on known chemical, biochemical, and biologic properties of nitrosoureas led to the testing of combinations of CCNU with chlorozotocin for in vivo activity against L1210 leukemia. Several combinations yielded synergistic antileukemic activity.

[Cancer Treat Rep 65:591–599, 1981]

It has been demonstrated (1,2) that some of the chemotherapeutically useful nitrosoureas have different chemical, biochemical, and biologic properties. It is reasonable to think that combinations of two or more nitrosoureas might be utilized to take advantage of the specific properties of each compound for therapeutic purposes. One such pair consists of CCNU and chlorozotocin.

CCNU has moderately high alkylating activity and high carbamoylating activity (1), and it is active against L1210 cells implanted ip, intracerebrally, and iv (2), but it causes undesirable marrow toxicity (3,4).

Chlorozotocin has high alkylating activity and very little carbamoylating activity (1). It causes very little marrow toxicity in mice (4), but it is not effective against intracerebrally implanted L1210 cells (unpublished data from this laboratory).

Available data (5) indicate that the anticancer activity of the *N*-(2-chloroethyl)-*N*-nitrosoureas is related to the alkylating activity, and there is also evidence that the carbamoylating activity is related to the inhibition of repair (6), perhaps because of deactivation of the DNA ligase (7).

It has been reported (8) that CCNU and chlorozotocin alkylated both the core particles and the internucleosomal regions of the chromatin of L1210 cells in vitro, but in murine marrow cells CCNU preferentially alkylated the core particles, while chlorozotocin preferentially alkylated the internucleosomal regions.

A combination of CCNU and chlorozotocin might have the following advantages: (*a*) Both agents might contribute to the total alkylating activity, but the total marrow toxicity might be less than if a dose of CCNU having the equivalent total alkylating activity were used alone. (*b*) The CCNU of the combination might be effective against L1210 cells that had infiltrated the brain, whereas chlorozotocin alone would be ineffective. (*c*) The carbamoylating activity of the CCNU might inhibit the repair of the damage to the DNA caused by the combined alkylating activities of the two agents. [However, in separate tests with streptozocin and with 3-β-D-glucopyranosyl-1-methyl-1-nitrosourea, the two compounds were about equally cytotoxic to L1210 cells, although only the latter compound retarded the repair of alkylation damage (9). (*d*) Since CCNU and chlorozotocin bind similarly to the chromatin of L1210 cells but differently to the chromatin of murine marrow cells, a combination of the two agents might have a greater cytotoxic effect on the leukemic cells than on the marrow cells. (*e*) As a result of the above possibilities, the combination might be effective against disseminated L1210, including cells that had infiltrated the brain, with a degree of marrow toxicity that could be tolerated.

The experiments reported here were performed to determine whether a combination of CCNU and

[1]Received June 4, 1980; revised Sept 15, 1980; accepted Sept 23, 1980.

[2]Supported by grant CH-100A from the American Cancer Society and by contract N01-CM-97309 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Biochemistry (*G. P. Wheeler*) and Chemotherapy (*F. M. Schabel, Jr,* and *M. W. Trader*) Departments, Southern Research Institute, Birmingham, AL.

**Reprint requests to:* Dr. Glynn P. Wheeler, Biochemistry Department, Southern Research Institute, 2000 9th Ave, Birmingham, AL 35255.

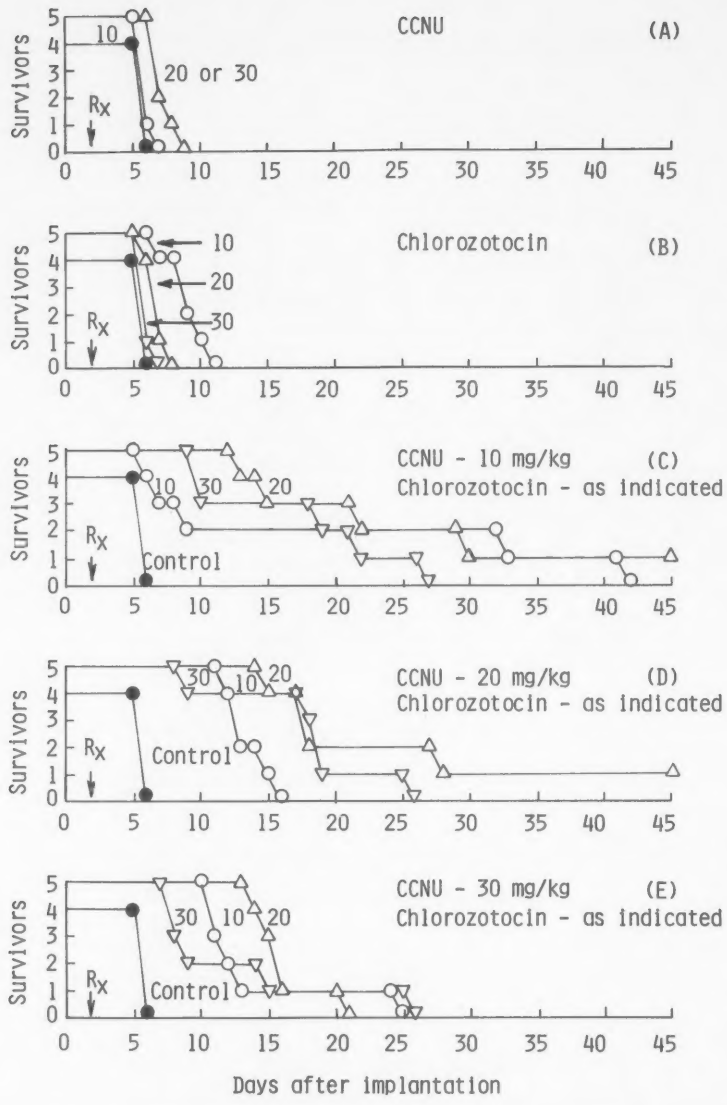

FIGURE 1.—Survival of leukemic mice treated with CCNU and chlorozotocin: experiment 1. Mice received 1×10^6 L1210 cells ip and single ip injections of the drugs 2 days later. In the combinations, the CCNU was administered 2 hrs before the chlorozotocin. The numbers beside the curves refer to dose in mg/kg. Each test group contained 5 mice; the control group contained 4 mice.

chlorozotocin would indeed result in therapeutic synergism against L1210 cells implanted ip.

MATERIALS AND METHODS

The CCNU and the chlorozotocin were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The CCNU was either suspended in 0.4% carboxymethyl cellulose in physiologic saline or dissolved in ethanol with one volume of Emulphor EL-620 (GAF Corporation, New York, NY) and eight volumes of physiologic saline added sequentially to the ethanol solution. The chlorozotocin was dissolved in physiologic saline. The agents were injected ip into mice within 15 minutes after they were prepared. In all experiments the agents were given as single doses on the basis of the exact weights of the mice. When both agents were given to the same mice, the CCNU was given 2 hours before the chlorozotocin. In experiments 1 and 2 the control mice and the mice that received only one agent received injections of the appropriate vehicles, so that all of the mice in the same experiment received the same levels of vehicles.

The L1210 ascites leukemia was carried in serial passage in CDF_1 mice with transfers on Day 7 following the implantation of 1×10^5 cells. For use in these experiments the cells were harvested on Day 7, diluted to the desired cell density with physiologic saline or with Fischer's cell culture medium containing 10% horse serum, and implanted ip into CDF_1 mice. The numbers of cells implanted are indicated in the figure legends and tables. The animals were observed daily for deaths, and the dead mice were autopsied to determine the presence of ascites and splenomegaly.

RESULTS AND DISCUSSION

Two of the four experiments presented here were performed in a laboratory of the Biochemistry Department, and the other two were performed in a laboratory of the Chemotherapy Department at this Institute. Different investigators performed the two pairs of experiments over a period of approximately 10 months.

In the experiment represented by figure 1, 1×10^6 cells/mouse were implanted in groups of five mice, and the drugs were administered 2 days later. Sections A and B of figure 1 show that either CCNU or chlorozotocin alone caused only slight increases in the median lifespans of the mice, without any long-term survivors. On the other hand, all of the combinations tested (sections C, D, and E of fig 1) caused much greater increases in median lifespans of the mice, and two of the mice were still alive at 45 days. To evaluate drug toxicity, the individual drugs and each combination were also administered at the indicated doses to mice that had not received implants of leukemic cells. At none of the dose levels did CCNU cause toxicity deaths, but one or more toxicity deaths occurred with a 30-mg/kg dose of chlorozotocin, whether it was given alone or in combination with CCNU. Since the definition for chemotherapeutic synergism that we followed (10) requires that a combination of drugs must have greater therapeutic effect than can be obtained by the individual drugs at the maximum tolerated doses, this experiment left open the possibility that larger doses of CCNU might individually yield increases in lifespans equal to or greater than those obtained with the combinations. Therefore, the second experiment was performed.

The second experiment (fig 2) was essentially the same as experiment 1 except that larger doses of the individual drugs were tested. CCNU doses of 70 and 60 mg/kg were toxic to the mice, but a dose of 50 mg/kg was tolerated, and at this dose, CCNU caused an approximate doubling of the median lifespan of the mice. Chlorozotocin at a dose of 35 mg/kg was toxic to the mice, but doses of 30 and 25 mg/kg were tolerated and caused an approximate doubling of the median lifespan. Although two of the combinations (10 mg/kg of CCNU + 30 mg/kg of chlorozotocin and 30 mg/kg of CCNU + 20 mg/kg of chlorozotocin) caused death from toxicity of one mouse in each group (LD20), the remaining mice that received the latter combination were alive at 45 days, and two of the remaining mice that received the former combination were alive at 45 days. Four of the five mice that received 10 mg/kg of CCNU + 20 mg/kg of chlorozotocin were alive at 45 days, and all of the mice that received 20 mg/kg of CCNU + 20 mg/kg of chlorozotocin were alive at 45 days. These results suggested chemotherapeutic synergism with the combination.

To test this combination further, experiments 3 (fig 3) and 4 (fig 4) were performed. These experiments were performed in another laboratory and by investigators other than those who performed experiments 1 and 2. In these experiments the number of animals in each drug-treated group was increased to ten, and the untreated leukemic control group contained 20 animals. The number of cells implanted was increased to 1×10^7, and treatment was delayed until 3 days after the implantation, at which time the body burden of viable tumor cells

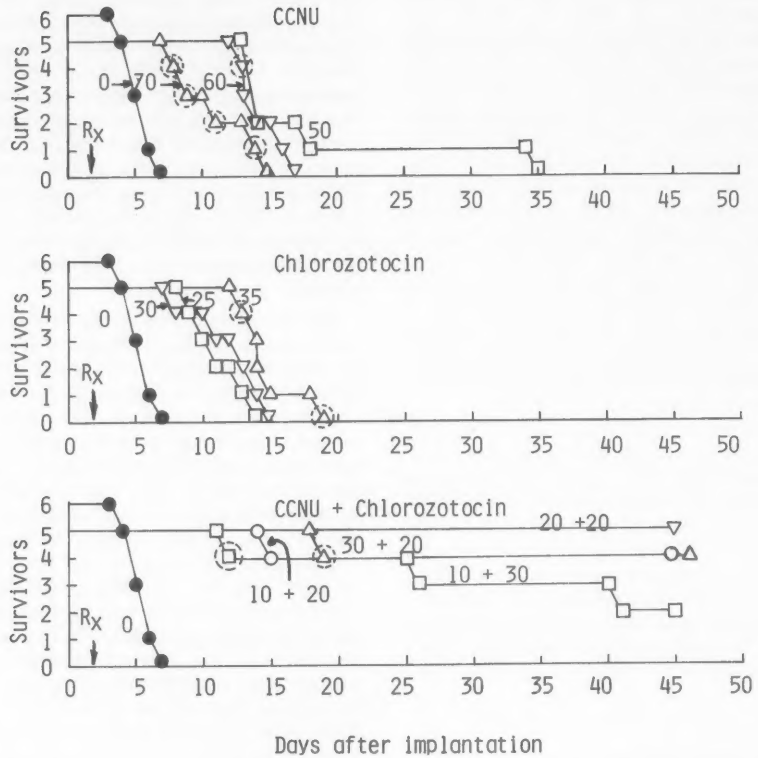

FIGURE 2.—Survival of leukemic mice treated with CCNU and chlorozotocin: experiment 2. Mice received 5.8×10^6 L1210 cells ip and single ip injections of the drugs 2 days later. In the combinations, the CCNU was administered 2 hrs before the chlorozotocin. The numbers beside the curves refer to the doses in mg/kg. A dashed circle around a symbol indicates that death was due to drug toxicity, which was recognized by subnormal spleen weight and the absence of ascites. The control group contained 6 mice; all other groups contained 5 mice.

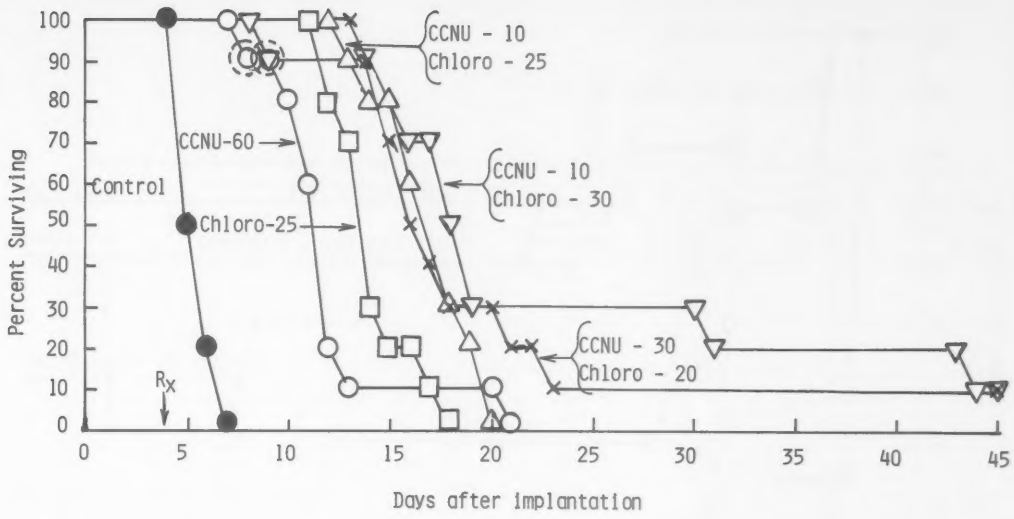

FIGURE 3.—Survival of leukemic mice treated with CCNU and chlorozotocin: experiment 3. Mice received 1×10^5 L1210 cells ip and single ip injections of the drugs 3 days later. In the combinations the CCNU was administered 2 hrs before the chlorozotocin (chloro). The numbers indicate the doses (mg/kg) of the respective drugs. A dashed circle around a symbol indicates that death was due to drug toxicity, which was recognized by subnormal spleen weight and the absence of ascites. In this experiment there were 20 animals in the control group; all other groups contained 10 animals. In this experiment the CCNU was prepared for injection by grinding in the carboxymethyl cellulose vehicle.

was estimated to exceed 1×10^8. In experiment 3 the CCNU was suspended in the carboxymethyl cellulose preparation, and in experiment 4 it was prepared in the ethanol-Emulphor-saline mixture. The complete data are presented in tables 1 and 2, but some of the data are presented in figures 3 and 4 to facilitate comparison with the data of figures 1 and 2. The data of experiments 3 and 4 are definitely indicative of chemotherapeutic synergism against advanced disease. In experiment 3 (table 1) few mice were cured by drug treatment, but the median lifespan of mice treated with CCNU plus chlorozotocin was markedly increased over that obtained with either drug alone. In experiment 4 (table 2) both the cure rates and the median lifespan of drug-treated animals dying of leukemia were consistently greater than those obtained at equitoxic doses of either drug alone.

A comparison of the data for experiments 2, 3, and 4 shows that there were considerable differences in the doses of CCNU that caused toxicity deaths of the animals. These differences might be at least partially due to the different ways in which the drug was prepared for injection. In experiment 2 the CCNU was prepared in the ethanol-Emulphor-saline system, and the resulting clear solution was injected into the mice. In experiment 3 the CCNU was "homogenized" in the carboxymethyl cellulose vehicle with a motor-driven Teflon-glass homogenizer to yield a nonuniform suspension. In experiment 4 the CCNU was dissolved in ethanol and Emulphor was added, but upon the subsequent addition of the saline to the clear solution, precipitation of fine particles of CCNU occurred, yielding what appeared to be a more uniform suspension of particles than that obtained in experiment 3. Other experiments performed in this laboratory have shown that two batches of CCNU having different particle sizes yielded different antileukemic activities when homogenized similarly in the carboxymethyl cellulose vehicle. The batch with the larger particle size had less activity, but after reprecipitation of the large-particle material to yield smaller particles, the activity increased. Therefore, in the present experiments it is reasonable to consider each experiment independently to detect synergism.

Although experiments were performed over several months by different persons in different labo-

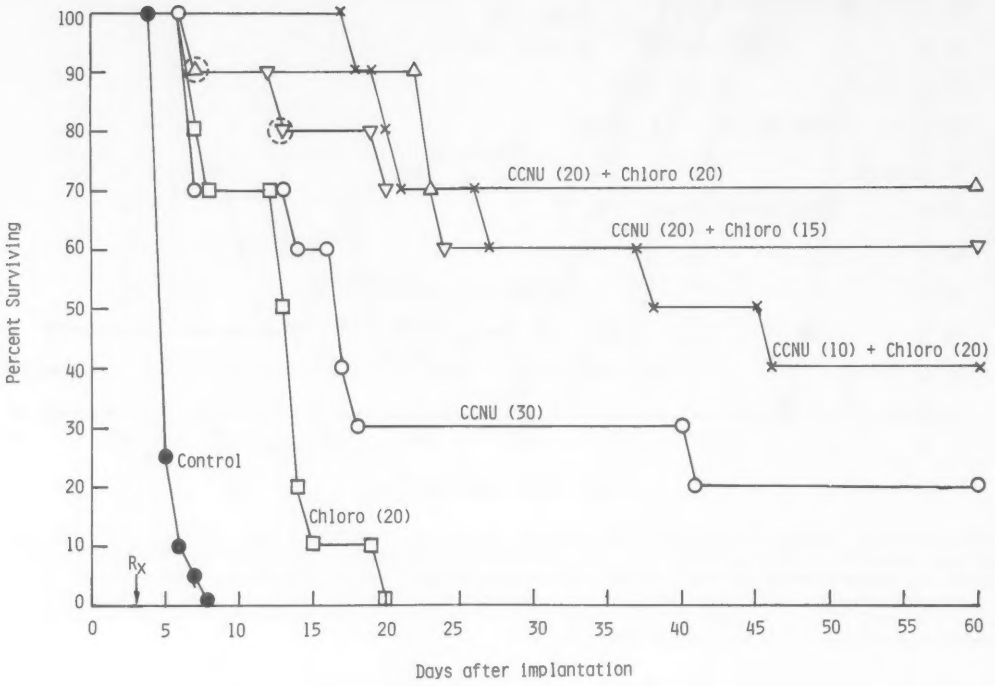

FIGURE 4.—Survival of leukemic mice treated with CCNU and chlorozotocin: experiment 4. Mice received ip implantations of 1×10^7 L1210 cells and single ip injections of the drugs 3 days later. In the combinations the CCNU was administered 2 hrs before the chlorozotocin (chloro). The numbers indicate the doses (mg/kg) of the respective drugs. A dashed circle around a symbol indicates that death was due to drug toxicity, which was recognized by subnormal spleen weight and the absence of ascites. The control group contained 20 animals; each other group contained 10 animals. In this experiment the CCNU was prepared for injection with the ethanol-Emulphor-saline vehicle.

ratories with different batches of drugs prepared in different ways for injection into mice bearing different body-burdens of L1210 cells, the results consistently indicate that in this experimental system a combination of two nitrosoureas provided greater therapeutic benefit than could be obtained with either agent alone. In all of these experiments, the CCNU was administered 2 hours prior to the administration of the chlorozotocin, because it was reasoned that it might be desirable to decrease the repair capacity of the cells (resulting from the carbamoylation caused by the CCNU) prior to subjecting the cells to the supplementary alkylating activity provided by the chlorozotocin. No other schedule was tested, and it is possible that other schedules would be equally or perhaps more effective.

These experiments neither confirm nor negate the biochemical logic set forth in the introduction, and the observed synergistic action may be due to some completely different reason(s). Nevertheless, a rational biochemical basis led to the performance of these experiments, which indicate that in addition to combinations of two types of alkylating agents being useful for therapy (12), combinations of two nitrosoureas might also be useful. On the basis of the available information on other nitrosoureas (1), other therapeutically useful combinations of nitrosoureas could also be devised.

TABLE 1.—Combination chemotherapy of advanced L1210 leukemia with CCNU plus chlorozotocin (experiment 3)

No. of L1210 cells implanted ip	Ip drug treatment (mg/kg)*	No. of toxicity deaths	Median day of death of dying mice	Median % increase in lifespan	No. of 45-day survivors	Approximate $\log_{10}$ tumor cell kill by drug†
10^7‡	None§	—	5.5	—	0	—
10^6		—	7.0	—	0	—
10^5		—	8.0	—	0	—
10^4			10.0	—	0	—
10^3		—	10.0	—	0	—
10^2		—	11.0	—	0	—
10		—	12.0	—	0	—
1		—	13.0	—	5	—
10^7	CCNU (70)	1	9.0	63	0	1.9
10^7	CCNU (60)	1	12.0	118	0	4.8
10^7	CCNU (50)	0	10.0	81	0	2.9
10^7	CCNU (45)	0	8.5	54	0	1.4
10^7	CCNU (40)	0	8.5	54	0	1.4
10^7	CCNU (30)	0	9.0	63	0	1.9
10^7	Chloro (40)	9	9.0	63	0	Toxic
10^7	Chloro (35)	6	11.5	109	0	Toxic
10^7	Chloro (30)	0	12.5	127	0	5.3
10^7	Chloro (25)	0	14.0	154	0	6.7
10^7	Chloro (20)	0	13.0	136	0	5.8
10^7	Chloro (10)	0	9.0	63	0	1.9
10^7	CCNU (30) + chloro (20)	0	16.0	190	1	≈8.4 (8.0)
10^7	CCNU (30) + chloro (10)	0	14.0	154	0	6.7
10^7	CCNU (20) + chloro (25)	1	15.5	181	0	8.2
10^7	CCNU (20) + chloro (20)	0	15.0	172	0	7.7
10^7	CCNU (20) + chloro (15)	0	13.0	136	0	5.8
10^7	CCNU (20) + chloro (10)	0	11.0	100	0	3.8
10^7	CCNU (10) + chloro (30)	1	18.0	227	1	≈8.4 (8.0)
10^7	CCNU (10) + chloro (25)	0	17.0	209	0	≈8.4
10^7	CCNU (10) + chloro (20)	0	16.0	190	0	≈8.4
10^7	CCNU (10) + chloro (15)	0	12.5	127	0	5.3
10^7	CCNU (10) + chloro (10)	0	10.5	90	0	5.3

*When a combination was given, the CCNU was given 2 hrs before the chlorozotocin (chloro). Unless specified otherwise, there were 10 mice/group.

†Based on the median day of death (dying mice only). Values in parentheses are based on % survivors, assuming that the No. of cells surviving treatment in each animal obeys a Poisson distribution and that those animals still alive at 45 days had no tumor cell that survived treatment (11).

‡This group had 20 mice.

§The untreated L1210 titration indicated a population doubling time of 0.31 day and a tumor burden at start of treatment of ≈2.5 × 10^8 L1210 cells/mouse.

TABLE 2.—Combination chemotherapy of advanced L1210 leukemia with CCNU plus chlorozotocin (experiment 4)

No. of L1210 cells implanted ip	Ip drug treatment (mg/kg)*	No. of toxicity deaths	Median day of death of dying mice	Median % increase in lifespan	No. of 60-day survivors	Approximate $\log_{10}$ tumor cell kill by drug†
10^7‡	None§	—	5.0	—	0	—
10^6		—	7.0	—	0	—
10^5		—	8.0	—	0	—
10^4		—	9.0	—	0	—
10^3		—	10.0	—	0	—
10^2		—	11.0	—	0	—
10		—	12.0	—	0	—
1		—	16.0	—	5	—
10^7	CCNU (70)	7	8.0	60	0	Toxic
10^7	CCNU (60)	10	9.0	80	0	Toxic
10^7¶	CCNU (50)	9	24.0	380	0	Toxic
10^7	CCNU (40)	7	25.0	400	0	Toxic
10^7	CCNU (30)	0	15.5	210	2	7.1 (8.2)
10^7	Chloro (35)	7	9.0	80	0	Toxic
10^7	Chloro (30)	6	10.5	110	0	Toxic
10^7	Chloro (25)	4	15.0	200	0	Toxic
10^7	Chloro (20)	0	13.5	170	0	5.6
10^7	Chloro (10)	0	9.0	80	0	2.2
10^7	CCNU (30) + chloro (20)	5	17.5	250	2	Toxic
10^7	CCNU (30) + chloro (10)	3	22.5	350	4	Toxic
10^7	CCNU (20) + chloro (25)	8	23.5	370	0	Toxic
10^7	CCNU (20) + chloro (20)	1	23.0	360	7	≈8.4 (8.9)
10^7	CCNU (20) + chloro (15)	2	16.5	230	6	7.8 (8.7)
10^7	CCNU (20) + chloro (10)	1	16.0	220	3	7.4 (8.4)
10^7	CCNU (10) + chloro (30)	3	15.0	200	0	Toxic
10^7	CCNU (10) + chloro (25)	1	16.0	220	2	7.4 (8.2)
10^7	CCNU (10) + chloro (20)	0	24.0	380	4	≈8.4 (8.5)
10^7	CCNU (10) + chloro (15)	0	18.0	260	0	≈8.4
10^7	CCNU (10) + chloro (10)	0	15.0	200	0	6.7

*When a combination was given, the CCNU group was given 2 hrs before the chlorozotocin (chloro). Unless specified otherwise, there were 10 mice/group.

†Based on the median day of death (dying mice only). Figures in parentheses are based on % survivors, assuming that the No. of cells surviving treatment in each animal obeys a Poisson distribution and that those animals still alive at 60 days had no tumor cells that survived treatment (11).

‡This group had 20 mice.

§The untreated L1210 titration indicated a population doubling time of 0.40 day and a tumor burden at start of treatment of ≈2.8 × 10^8 L1210 cells/mouse.

¶This group had 9 mice.

REFERENCES

1. WHEELER GP. A review of studies on the mechanism of action of nitrosoureas. *In* Cancer Chemotherapy (Sartorelli AC, ed). American Chemical Society Symposium, 1976, Series 30, pp 87–119.
2. SCHABEL FM, JR. Nitrosoureas: a review of experimental antitumor activity. Cancer Treat Rep 60:665–698, 1976.
3. SCHEIN PS, MCMENAMIN MG, and ANDERSON T. 3-(Tetra-acetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea, an antitumor agent with modified bone marrow toxicity. Cancer Res 33:2005–2009, 1973.
4. ANDERSON T, MCMENAMIN MG, and SCHEIN PS. Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose, an antitumor agent with modified bone marrow toxicity. Cancer Res 35:761–765, 1975.
5. WHEELER GP, BOWDON BJ, GRIMSLEY JA, ET AL. Interrelationships of some chemical, physicochemical, and biological activities of several 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 34:194–200, 1974.
6. KANN HE, JR, SCHOTT MA, and PETKAS A. Effects of structure and chemical activity on the ability of nitrosoureas to inhibit DNA repair. Cancer Res 40:50–55, 1980.
7. FORNACE AJ, JR, KOHN KW, and KANN HE, JR. Inhibition of the ligase step of excision repair by 2-chloroethyl isocyanate, a decomposition product of 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 38:1064–1069, 1978.
8. GREEN D, SMULSON ME, and SCHEIN PS. Differential binding of chlorozotocin (Clz) and CCNU to murine bone marrow chromatin. Proc Am Assoc Cancer Res and ASCO 20:253, 1979.
9. HEAL JM, FOX PA, and SCHEIN PS. Effect of carbamoylation on the repair of nitrosourea-induced DNA alkylation damage in L1210 cells. Cancer Res 39:82–89, 1979.
10. VENDITTI JM, HUMPHREYS SR, MANTEL N, ET AL. Combined treatment of advanced leukemia (L1210) in mice with amethopterin and 6-mercaptopurine. JNCI 17:631–638, 1956.

11. SCHABEL FM, JR, GRISWOLD DP, JR, LASTER WR, JR, ET AL. Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol Ther 1:411–435, 1977.
12. SCHABEL FM, JR, TRADER MW, LASTER WR, JR, ET AL. Patterns of resistance and therapeutic synergism among alkylating agents. Fundamentals in cancer chemotherapy. Antibiot Chemother 23:200–215, 1978.

Effect of Chemotherapy for the Dysmyelopoietic Syndrome[1,2]

James O. Armitage,* Fred R. Dick, Samuel W. Needleman, and C. Patrick Burns[3]

SUMMARY

Twenty patients are described who received chemotherapy for the diagnosis of acute myeloid leukemia but who were subsequently reclassified as having dysmyelopoietic syndrome using the French-American-British criteria. Thirteen patients had refractory anemia with excess blast cells (RAEB) (age range, 23–82 years; median, 68) and seven had chronic myelomonocytic leukemia (CMML) (age range, 44–79 years; median, 70). Three patients (two with RAEB and one with chronic myelomonocytic leukemia) had previously been treated with cytotoxic therapy for another malignancy. In 15 patients the antileukemic therapy was a chemotherapy regimen that was highly active in acute nonlymphoblastic leukemia (ie, containing daunorubicin and/or cytarabine). These patients achieved complete remission lasting 14, 34+, and 36+ months and survival times of 31, 35+, and 37+ months. All three patients were from a subgroup of four patients characterized by RAEB, younger age, no previous cytotoxic therapy, and treatment with an aggressive chemotherapy regimen. The median survival time of the patients not achieving remission was 1 month (range, < 1–22). Our results suggest that while most patients with the dysmyelopoietic syndrome appear to have their life shortened by chemotherapy, there is a subgroup characterized by younger age, absence of previous cytotoxic therapy, and the morphologic picture of RAEB who can have a favorable response to aggressive treatment.

[Cancer Treat Rep 65:601–605, 1981]

The French-American-British classification of acute leukemia includes the description of two dysmyelopoietic (or myelodysplastic) syndromes, refractory anemia with excess blast cells (RAEB) and chronic myelomonocytic leukemia (CMML) (1). It has been suggested that patients in these categories tolerate chemotherapy poorly, and that treatment should be withheld until overt leukemia develops (2). Twenty patients who were originally diagnosed as having acute leukemia and were treated with chemotherapy at the University of Iowa Hospitals were reclassified as having dysmyelopoietic syndrome on review of the marrow smears. The results of therapy suggest that it might be possible to identify a subgroup of the dysmyelopoietic syndrome in which chemotherapy can be beneficial.

PATIENTS AND METHODS

The marrow smears of 235 patients who had acute leukemia diagnosed at the University of Iowa Hospitals between January 1973 and September 1978 were reviewed independently by two hematologist-oncologists (J. Armitage and C. Burns) and one hematopathologist (F. Dick) and were reclassified us-

[1]Received Nov 6, 1979; revised Oct 21, 1980; accepted Oct 22, 1980.

[2]Supported in part by the Iowa Leukemia and Cancer Research Fund. C. P. Burns is the recipient of Public Health Service Career Development Award CA-00324 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine (*J. O. Armitage, S. W. Needleman,* and *C. P. Burns*) and Pathology (*F. R. Dick*), University of Iowa College of Medicine, Iowa City.

**Reprint requests to:* James O. Armitage, MD, Department of Medicine, University of Iowa Hospitals, Iowa City, IA 52242.

ing the French-American-British criteria (1). Wright's stain, peroxidase, and PAS were used routinely and nonspecific esterase, methyl green pyronin, and serum lysozyme were used in selected cases. Acute lymphoblastic leukemia was separated from acute nonlymphoblastic leukemia on the basis of a negative peroxidase (ie, < 3% positivity in leukemic blast cells). The dysmyelopoietic syndrome was separated from acute nonlymphoblastic leukemia when the percent of immature leukemic cells on marrow differential was < 30%. Forty patients were reclassified as having the dysmyelopoietic syndrome. All of these patients had normal (four patients) or increased (36 patients) marrow cellularity for age, increased blast cells and promyelocytes in the marrow, decreased numbers of circulating normal cells, and no other identifiable cause. Patients with an absolute circulating monocyte count of $\geq 2.0 \times 10^9$/liter were classified as having CMML and the remainder were classified as having RAEB (fig 1).

Twenty of the 40 patients received chemotherapy because of a decision by their physician. All patients except two were treated promptly when the hematologic diagnosis was made. One 73-year-old male (who did not achieve remission) was followed for 15 months and then treated for worsening anemia, and another 68-year-old man (who did achieve remission) was followed for 8 months and then treated because of bleeding. Bone marrow smears at the time treatment was administered have been reclassified as dysmyelopoietic syndrome. Both patients had 28% blast cells and promyelocytes in the marrow at the time of chemotherapy. These 20 treated patients form the basis for this report. An additional 15 patients were followed without therapy because of a decision by their attending physician. Three of these were subsequently treated with chemotherapy after progressing to overt acute leukemia. Two of the 40 patients had received therapy before being seen at Iowa, making interpretation of their marrow pictures difficult, and three were Philadelphia chromosome-positive and probably had chronic myelocytic leukemia in transition (fig 1).

The criteria for complete remission (CR) were those of the acute leukemia group B.[4] The duration of CR is defined as the time from first achievement until the marrow is no longer M_1. The duration of survival is defined as the time from the initiation of therapy to death. Survival curves were prepared using the life-table method. Treatment regimens used were those chosen by the patient's attending

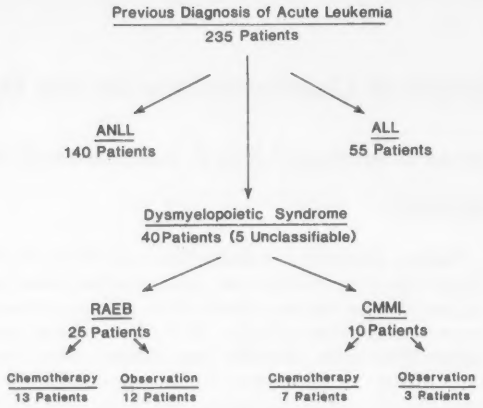

FIGURE 1.—Morphologic classification and eventual management in 235 patients first seen at the University of Iowa Hospitals between 1973 and 1978. ANLL = acute nonlymphoblastic leukemia; ALL = acute lymphoblastic leukemia.

physician and were varied. The doses of the four most commonly used regimens are as follows: 100 mg/m² of cytarabine iv and 100 mg/m² of 6-thioguanine orally, each given every 12 hours to marrow hypocellularity (AT regimen); 2 mg/kg of 6-thioguanine orally every 12 hours for 7 days, 2 mg/kg of cytarabine iv every 12 hours for 7 days, 1.5 mg/kg of daunorubicin iv daily for 3 days, 1 mg/kg of prednisone orally daily for 7 days, and 1 mg/m² of vincristine iv on Days 1 and 7 (TADPO regimen); 1 mg/kg of daunorubicin iv daily for 5 days and 60 mg of prednisone orally daily for 5 days (DP regimen); and 30 mg/m² of doxorubicin iv daily for 3 days, 100 mg/m² of cytarabine iv every 12 hours for 7 days, and 100 mg/m² of 6-thioguanine orally every 12 hours for 7 days (HAT regimen).

RESULTS

The clinical data, hematologic parameters, and treatment outcome for the 20 patients treated for dysmyelopoietic syndrome are listed in tables 1 and 2. Thirteen patients had RAEB (age range, 23–82 years; median, 68) and seven had CMML (age range, 44–79 years; median, 70). Three patients (two with RAEB and one with CMML) had received previous cytotoxic therapy for another malignant disorder. Patient 4 had been treated with chemotherapy and radiation therapy for Hodgkin's disease, patient 6 had been treated with chemotherapy and radiation therapy for ovarian carcinoma, and patient 10 had

[4] Acute Leukemia Group B. Criteria for evaluating acute leukemia of the Acute Leukemia Group B. June 1974 modification of the 1969 criteria. Cancer and Leukemia Group B, Scarsdale, NY.

TABLE 1.—Hematologic parameters in patients with the dysmyelopoietic syndrome

Patient No.	Peripheral blood (Cells × 10^9/liter) Wbcs	Mature neutrophils	Monocytes	Blast cells	Platelets	Hemoglobin (g/100 ml)	Marrow % blast cells	% promyelocytes	Auer rods	Ringed sideroblasts
				Patients achieving CR						
1	5.5	3.2	0.3	0.7	88	7.3	11	8	+	+
2	1.0	0.2	0.2	0.1	48	6.7	10	9	–	–
3	1.8	0.1	0.3	0	10	8.5	12	16	–	–
				Patients not achieving remission						
4	4.0	0.2	0.1	0.7	20	4.9	21	4	+	+
5	25.7	11.0	3.6	3.3	25	9.0	20	3	–	–
6	3.4	0.9	0.5	0	20	5.7	11	10	–	+
7	4.4	1.9	0.7	0.1	155	9.2	10	2	–	–
8	2.9	1.1	0.2	0	161	10.9	10	17	–	–
9	27.0	10.0	8.1	0	95	11.1	6	1	–	–
10	43.5	10.4	11.2	3.5	10	9.1	5	20	–	+
11	97.0	46.6	5.8	30.1	36	7.9	20	4	–	–
12	2.8	1.7	0.1	0.1	27	6.6	10	8	–	+
13	3.0	2.4	0.1	0	7	8.6	12	5	–	–
14	30.1	2.4	2.6	3.0	11	4.7	14	3	+	–
15	76.5	33.6	7.7	0	8	11.8	13	7	–	–
16	2.3	1.1	0.1	0	2	8.2	20	8	–	+
17	8.5	2.9	0.7	0.1	4	5.1	18	10	–	+
18	2.7	1.8	0.1	0	16	9.7	10	13	–	–
19	28.1	18.1	2.2	2.5	81	9.7	4	16	–	–
20	3.2	0.2	0.1	0.3	83	12.5	14	8	+	–

received both chemotherapy and radiation therapy for a nodular lymphocytic lymphoma. The treatment regimens used for remission induction in acute leukemia during the 6-year period varied. Fifteen patients were treated with a regimen that included drugs with a high activity in acute nonlymphoblastic leukemia (ie, daunorubicin and/or cytarabine). Five patients received less active regimens including vincristine and prednisone in two patients, 6-mercaptopurine and prednisone in one, hydroxyurea and prednisone in one, and busulfan alone in one.

The survival of the 20 treated patients is presented in figure 2. Three patients achieved CR. These patients were 23, 48, and 68 years of age, all had RAEB, and none had received previous cytotoxic therapy. Each of the three patients had significant symptoms from cytopenias. In each case the remission was induced with daunorubicin- and/or cytarabine-containing regimens (table 2). The CRs were maintained with intermittent courses of cytarabine and 6-thioguanine as previously described (3). The CR durations were 14, 34+, and 36+ months and the patients have survived 31, 35+, and 37+ months from beginning therapy.

The 17 nonresponding patients survived from < 1 to 22 months, with a median survival of 1 month. The effects of chemotherapy in the 12 nonresponding patients who were treated aggressively included four patients (Nos. 4, 12, 14, and 20) who had no reduction in marrow cellularity, three patients (Nos. 5, 6, and 8) who had marrow hypoplasia followed by regrowth of leukemia, three patients (Nos. 10, 13, and 18) who died with hypocellular marrows, and two patients (Nos. 16 and 17) who died before a repeat bone marrow examination was done to assess treatment response (table 2). No patient with CMML, with age > 70 years, with previous cytotoxic therapy for another malignancy, or with treatment other than aggressive antileukemic therapy achieved CR.

The patients who were not treated with chemotherapy were similar to the treated patients except for a lower mean wbc count, a higher mean platelet count, and a tendency to lower marrow cellularity in the untreated patients. The difference in wbc counts reflects the lower proportion of CMML in the untreated patients. The survival times of the untreated patients are contrasted to the treated group in figure 2. Treated patients died rapidly if CR was not obtained, in contrast to the slower death of untreated patients. Table 3 contrasts the treated and untreated groups by subtype of dysmyelopoietic syndrome. The only CRs (and prolonged symptom-free survival) were in treated patients with

TABLE 2.—Results of chemotherapy for the dysmyelopoietic syndrome

Patient No.	Age (yrs)	Sex	Previous cytotoxic therapy	Dysmyelopoietic syndrome subclass	Chemotherapy regimen*	Duration of CR (mos)	Survival from therapy (mos)
				Patients achieving CR			
1	23	M	–	RAEB	DP	14	31
2	48	M	–	RAEB	TADPO	36+	37+
3	68	M	–	RAEB	AT	34+	35+
				Patients not achieving remission			
4	34	M	+	RAEB	HAT	–	1
5	44	F	–	CMML	AT, D	–	22
6	46	F	+	RAEB	TADPO	–	6
7	53	M	–	RAEB	6-Mercaptopurine and P	–	< 1
8	63	F	–	RAEB	AT	–	1
9	63	F	–	CMML	Busulfan	–	2
10	69	F	–	CMML	AT	–	< 1
11	70	M	–	CMML	PO	–	2
12	70	M	–	RAEB	AT	–	9
13	71	M	–	RAEB	HAT	–	1
14	72	F	–	CMML	DP	–	< 1
15	73	M	–	CMML	Hydroxyurea and P	–	1
16	73	M	–	RAEB	DP	–	< 1
17	73	M	–	RAEB	AT	–	< 1
18	78	M	–	RAEB	OAP	–	1
19	79	M	–	CMML	PO	–	1
20	82	M	–	RAEB	AT	–	1

*D = daunorubicin, P = prednisone, T = 6-thioguanine, A = cytarabine, O = vincristine, H = doxorubicin.

RAEB and the longest median survival was in the untreated patients with RAEB. Only one patient with CMML survived > 2 months, irrespective of therapy.

DISCUSSION

Although the overall response rate is low, it appears that a subgroup of patients with the dysmyelopoietic syndrome can benefit from aggressive chemotherapy. This study suggests that the characteristics of that subgroup include relatively younger age, the absence of previous cytotoxic therapy, and the morphologic picture of RAEB. Three of four patients with those characteristics who were treated with drugs highly active in acute nonlymphoblastic leukemia became clinically and hematologically well for significant periods. Younger age and absence of previous cytotoxic therapy are also good prognostic factors in acute nonlymphoblastic leukemia.

It is important to identify responsive subgroups of the dysmyelopoietic syndrome, not just for their benefit, but so that treatment may be withheld in the other patients in whom it may be deleterious. Dreyfus (4), combining both his patients and those reported in the literature, reported a mean (median not given) survival of 20 months for patients with RAEB. He also described seven patients with RAEB who received treatment with regimens including daunorubicin and/or cytarabine (4). Two of the seven patients achieved CRs lasting 12 and 3+ months. Both responding patients were < 40 years of age. Izrael et al (5) described 11 patients with < 50% blastcells and promyelocytes in the marrow who

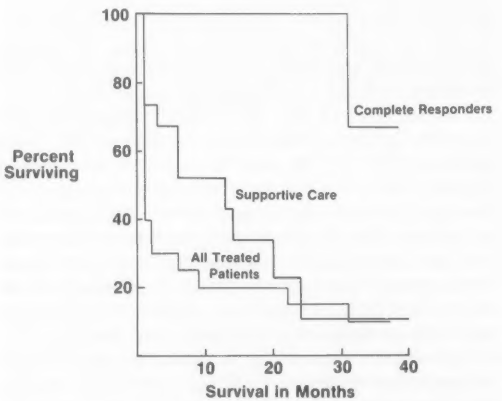

FIGURE 2.—Life-table plot of the survival from therapy of 20 patients with the dysmyelopoietic syndrome who were treated with chemotherapy, 3 of the treated patients who achieved a CR, and 15 patients who were managed with supportive care.

TABLE 3.—Outcome for patients by subtype of dysmyelopoietic syndrome and type of management

	No. of patients	Age in yrs (range)	No. of patients with previous cytotoxic therapy	No. of CRs	Survival in mos (range)
RAEB					
Treated	13	68 (23–82)	2	3	1 (<1–37+)
Untreated	12	72 (54–83)	0	—	14 (1–42+)
CMML					
Treated	7	70 (44–89)	1	0	1 (<1–22)
Untreated	3	72 (50–85)	2	—	1 (<1–1)

were treated with aggressive chemotherapy (5). Although the exact response rates are not given, the longest survival time was 16 months. Our results are in general agreement with these previous findings. We found a 14-month median survival time in untreated patients with RAEB that seemed to be markedly shortened by chemotherapy. The exception was found in the younger patients who were not treated with cytotoxic agents before developing RAEB; three of four of these patients achieved CR. Survival was short in patients with CMML whether or not they had received chemotherapy.

It is known that some patients with the dysmyelopoietic syndrome will progress to overt acute leukemia when followed without therapy (4). It is possible that the patients responding to chemotherapy are those who actually have acute leukemia but have not accumulated a sufficient tumor burden to enable certain diagnosis. Against this interpretation is the absence of complete responders in CMML, a disorder that has a higher rate of progression to overt acute leukemia than RAEB (2). The observation in this study that more than one half of the 13 evaluable, aggressively treated patients had abnormal cells that either were refractory to chemotherapy (four patients) or regrew after initial cytoreduction (three patients) suggests that if this is "early" acute leukemia it is perhaps more refractory to chemotherapy than "advanced" leukemia. At present, it is not certain whether or not the responders are patients with acute leukemia who were treated early in their disease.

A small proportion of patients with the dysmyelopoietic syndrome have a favorable response to chemotherapy and achieve prolonged, symptom-free survival. The results of this study suggest that the responders belong to a subgroup of patients with the morphologic picture of RAEB who are younger and have not been previously treated with cytotoxic chemotherapy. The achievement of CR requires therapy with aggressive regimens that are highly active in acute nonlymphoblastic leukemia. It is possible that utilization of techniques such as bone marrow culture studies and chromosome banding in addition to clinical parameters to define subgroups, might improve selectivity. Identification of a subgroup of patients with dysmyelopoietic syndrome who benefit from aggressive chemotherapy is important so that the potential responders can be treated and chemotherapy can be withheld in patients where a deleterious effect is likely.

REFERENCES

1. BENNETT JM, CATOVSKY D, DANIEL MT, ET AL. Proposals for the classification of the acute leukemias. Br J Haematol 33:451–458, 1976.
2. SULTAN AC. Dysmyelopoietic syndromes. Classification of acute leukemia. Ann Intern Med 87:740–753, 1977.
3. ARMITAGE JO, and BURNS CP. Maintenance of adult acute nonlymphoblastic leukemia: an argument against the need for central nervous system prophylaxis. Cancer 41:697, 1978.
4. DREYFUS B. Preleukemic states. Blood Cells 2:33–45, 1976.
5. IZRAEL V, JACQUILLAT C, CHASTANG C, ET AL. Données nouvelles sur les leucémies oligo-blastiques. Nouv Presse Med 4:947–952, 1975.

Chromosome Analyses of Nurses Handling Cytostatic Agents[1]

H. Waksvik,* O. Klepp, and A. Brøgger[2,3]

SUMMARY

A cytogenetic study of ten nurses handling cytostatic agents (average exposure, 2150 hours) and ten female hospital clerks revealed an increased frequency of chromosome gaps and a slight increase in sister chromatid exchange frequency among the nurses. The increase may be due to exposure to cytostatic drugs and points to these agents as a possible occupational health hazard. A second group of 11 nurses handling cytostatic agents for a shorter period of time (average exposure, 1078 hours), and three other groups (eight nurses engaged in therapeutic and diagnostic radiology, nine nurses engaged in anesthesiology, and seven nurses in a postoperative ward) did not differ from the office personnel, except for an increased frequency of chromosome gaps in the radiology group.

[Cancer Treat Rep 65:607–610, 1981]

Many cytostatic agents used in cancer chemotherapy are known to be mutagens and carcinogens. The possible induction of secondary neoplastic disease as a result of cytostatic therapy is now being discussed (1–5). The handling of these substances by nurses and doctors may represent an occupational health hazard. One way of assessing a possible exposure to mutagenic/carcinogenic agents in humans is the analysis of chromosome damage (6) and sister chromatid exchange (7), although the method has its limitations. Increased chromosome damage and sister chromatid exchange have been observed after cytostatic therapy (8–12), but there are cases in which no such increases have been found (9,13). Thus, a positive finding is certainly significant, but a negative chromosome finding does not rule out a previous exposure to chemical mutagens.

During recent years an increasing number of cancer patients at the Norwegian Radium Hospital have been treated with cytostatic drugs. To evaluate the possible occupational health hazard of handling cytotoxic agents, we have studied the chromosomes of two groups of nurses who had worked daily with such chemicals.

MATERIALS AND METHODS

Ten nurses (group 1) were selected because of long-term handling of cytostatic drugs (final preparation of solutions and drawing solutions into syringes ready for infusion). Four of them work in the outpatient department, and the others work in the wards. The average estimated time of handling drugs was 2150 hours. All of the nurses claimed that they had used hand gloves and surgical masks all the time, whereas a fume cabinet had been used by only seven of them during the previous 10 months. Each nurse was asked to rank the ten cytostatic agents most frequently handled. Pooling this information gave the following ranking list: cyclophosphamide, vincristine, doxorubicin, methotrexate, vinblastine, mechlorethamine, 5-FU, dactinomycin, dacarbazine, bleomycin, cisplatin, lomustine (capsules), ifosfamide, abrin, VP-16-213, thiotepa, 9-hydroxyellipticine, mithramycin, procarbazine (capsules), cytabarine, and AMSA. One nurse had worked for 2½ years in orthopedic operating rooms, where she was exposed to anesthetic gases and had assisted

[1]Received Jan 14, 1980; revised Sept 9, 1980; accepted Sept 18, 1980.

[2]Norsk Hydro's Institute for Cancer Reserach (*H. Waksvik* and *A. Brøgger*) and The Norwegian Radium Hospital (*O. Klepp*), Oslo.

[3]We thank the nurses and clerks for their cooperation, Berit Sandstad for statistical analyses, and Reidun Norum for skillful technical assistance.

**Reprint requests to*: H. Waksvik, Norsk Hydro's Institute for Cancer Research, Montebello, Oslo 3, Norway.

at frequent roentgenographic examinations. The other nurses had never worked with diagnostic roentgenography or radiotherapy or in operating rooms.

As controls, we selected ten women (group 2) working as clerks in the same building.

After the first positive findings, a second group of 11 nurses (group 3) was selected for study. Most of them had handled cytostatic agents for a shorter period of time than the first group (average estimated handling time, 1078 hours). Furthermore, the study was extended with one group of eight nurses (group 4) engaged in therapeutic and diagnostic radiology, one group of nine nurses (group 5) exposed to the anesthetic gases halothane, enflurane, and nitrous oxide in operating rooms, and one group of seven nurses (group 6) working in a postoperative ward in which exposure to mutagenic agents is almost absent—five of them had occasionally handled a few cytostatic agents (cyclophosphamide, methotrexate, 5-FU, vincristine, and doxorubicin; average exposure, 109 hours).

All the subjects were healthy and free of any virus disease when the blood sample was drawn. The number of subjects reporting various, previous diagnostic roentgenographic examinations were approximately equal in the six groups (table 1). The total number of reported roentgenographic examinations did not appear to be different in any group. None of the persons had received any therapeutic irradiation. The occasional use of drugs was not different among the first five groups; none in group 6 reported any drug intake. Smoking habits were equally distributed among the groups, the few smokers being moderate consumers (average, seven cigarettes/day).

Whole-blood cultures were established in 50-ml Nunclon culture bottles standing upright and containing 3 ml of Ham's F-10 medium with 20% fetal bovine serum, 0.4 ml of heparinized blood, and 10 µl of phytohemmagglutinin P (Difco). For the study of chromosome damage, the cultures were harvested at 48 hours, and the air-dried chromosome preparations were stained in 2% Giemsa. One hundred metaphases were selected from each subject and scored for gaps, breaks, and exchanges. An aberration was scored as a break only when the distal part of the chromatid was dislocated; otherwise, it was scored as a gap (6). For the analysis of sister chromatid exchange (SCE), 5-bromo-2′-deoxyuridine (Sigma) was added after 24 hours in a concentration of 5 µg/ml. The cultures were harvested at 72 hours, and the slides were stained for 20–30 minutes in 2% Giemsa in 0.3 M Na_2HPO_4 at pH 10.4. The number of SCEs were counted in 30 cells from each subject. The chromosome analyses were made blindly within each of the two sequences of the study.

RESULTS

The results of the chromosome analyses are given in table 2. Statistical analysis with the Kruskal-Wallis test (14) shows that there are differences between the groups, both for gaps and SCEs ($P < 0.01$). The Wilcoxon two-sample, rank-sum test (14) was performed to compare the groups two by two. There are significantly more gaps in group 1 than in groups 2, 3, 5 ($P < 0.01$), and 6 ($P < 0.05$); the SCE frequency is significantly higher in group 1 than in all the other groups ($P < 0.01$); and the SCE frequency in group 2 is higher than in group 3 ($P < 0.05$) and group 6 ($P < 0.01$). We found no relationship in the individual cases between estimated accumulated time of exposure and frequency of gaps or SCEs.

DISCUSSION

Environmental factors such as viral disease, smoking, diagnostic and therapeutic irradiation, and drug intake may influence the chromosomal parameters. Care has been taken to evaluate these factors (table 1). The groups did not appear to be different in any of these respects, with the exception of less use of occasional drugs in group 6. The mean age is not the same in all of the groups. Ranking for age does not give the same sequence as rank-

TABLE 1.—Comparison of groups for factors which may influence chromosome parameters

Group No.	Type of work	No. of subjects	Mean age (yrs)	No. of smokers	No. reporting diagnostic roentgenograms	No. reporting occasional drug intake
1	Handling cytostatic agents	10	45.1	3	8	5
2	Hospital clerks	10	43.6	2	6	4
3	Handling cytostatic agents	11	36.7	1	8	4
4	Therapeutic/diagnostic radiology	8	45.8	2	5	4
5	Anesthesiology	9	35.4	2	7	3
6	Postoperative ward	7	36.0	2	6	0

TABLE 2.—Chromosome analyses of hospital personnel

Group No.	Type of work	Employment (yrs)	Estimated exposure (hrs)	Chromosome damage (per 100 cells) Gaps	Breaks	SCEs/cell	SD
1	Handling cytostatic agents	4.9	2150	18.5	0.8	8.5	3.6
2	Hospital clerks			9.3	0.3	6.5	2.9
3	Handling cytostatic agents	7.0	1078	7.4	0.7	5.8	2.5
4	Therapeutic/diagnostic radiology	6.4		13.9	1.0	6.2	3.0
5	Anesthesiology	3.6		7.8	1.6	6.1	2.6
6	Postoperative ward	4.9		10.3	0.6	5.1	1.8
Group 1 repeated after 14 months (6 subjects):				12.7	0.3	5.3	2.3

ing for SCE frequency, and the age difference between groups 1 and 2 is small. Age dependency of SCE frequency has been found. Ardito et al (15) reported a lower SCE frequency in newborn children than in their mothers, and in a series of female twins, Waksvik et al[4] observed a higher SCE frequency in a group 57–61 years old than in a group aged 33–39. In our material it seems reasonable to assume that differences in the chromosomal parameters are due to the working conditions.

The first group of nurses showed significantly higher frequencies of chromatid gaps and SCEs than the control group of office personnel. This indicates exposure to mutagenic agents, and it seems reasonable to suspect that these agents are the cytostatic drugs. Each of the nurses had handled many of the established and investigational drugs used in a large oncologic hospital. Using the bacterial fluctuation test, Falck et al (16) demonstrated increased mutagenicity of the urine of nurses handling cytostatic drugs, and later the Helsinki group revealed a slight increase in SCE frequency among 20 such nurses (17).

The chromatid gap in itself may not represent any serious chromosome damage, possibly just a transient folding defect of the chromosome fiber (18), but an increase in the gap frequency indicates exposure to mutagenic agents (19,20). It is quite unusual, however, to find such an increase without a concomitant increase of the chromatid break frequency.

The nurse in group 1 who had worked in orthopedic operating rooms presented with an exceptionally high number of gaps, 39%. She is the only person in her group who has been occupationally exposed to anesthetic gases, but it is doubtful whether this has any relationship to the number of gaps found. Anesthetic gases induce mitotic disturbances, not chromatid gaps, breaks, or exchanges (21,22). On the other hand, this nurse was presumably also exposed to ionizing radiation in her work, and gaps are induced by radiation (23).

The positive findings in group 1 were not confirmed in the second group of nurses handling cytostatic agents (group 3). The average estimated accumulated time of exposure was only one half as long in this group, 1078 hours vs 2150 in group 1. This may account for the difference. Group 3 was examined 6 months after the positive findings in the first group were known among the nurses in the hospital. It is possible that this information led to even more cautious handling of the drugs and that this is responsible for the normal chromosome findings in group 3. This interpretation is further supported by the findings in a repeated examination of six subjects in group 1 after 14 months. The SCE frequency was no longer increased.

The frequency of gaps was significantly increased among the nurses engaged in therapeutic and diagnostic radiology. This may be due to radiation exposure, since gaps are induced by radiation (23). On the other hand, the gap frequency is slightly increased among the nurses in the postoperative ward, suggesting that hospital work in general may be responsible in some way.

The nurses exposed to anesthetic gases did not differ from the control group. This is in accordance with the results reported by Husum and Wulf (24), who did not find any increase in SCE frequency among female nurses and male physicians exposed to halothane and nitrous oxide. It has also been shown that in vitro exposure of Chinese hamster cells to halothane, enflurane, and nitrous oxide in the presence of a metabolic activation system did not increase the SCE frequency (25).

The lowest SCE frequency was found in the postoperative ward group; it was significantly lower than in the control group. This may be fortuitous, but none of the nurses in group 6 reported any occasional use of drugs.

[4]Waksvik H, Magnus P, and Berg K. The effects of age, sex and genes on sister chromatid exchange. Clin Genet, 1981. In press.

Gap and SCE frequencies in the total material from the 12 smokers are not higher than in material from the nonsmokers in groups 2, 4, 5, and 6. Thus, we are not able to confirm that smoking increases the frequencies of chromosome damage (26) and SCE (27).

We found a very low frequency of chromatid breaks, no chromosomal exchange, and only a comparatively small increase of SCE in the blood cultures of the nurses most exposed to cytostatic agents. This indicates that their exposure to mutagenic agents must be fairly low. Still, our findings point to the handling of cytostatic drugs as a possible health hazard and indicate that protective measures (gloves, masks, and fume cabinets) should be used.

REFERENCES

1. Casciato DA, and Scott JL. Acute leukemia following prolonged cytotoxic agent therapy. Medicine 58:32–47, 1979.
2. Foucar K, McKenna RW, Bloomfield CD, et al. Therapy-related leukemia. A panmyelosis. Cancer 43:1285–1296, 1979.
3. Krikorian JG, Burke JS, Rosenberg SA, et al. The occurrence of non-Hodgkin's lymphoma following therapy for Hodgkin's disease. N Engl J Med 300:452–458, 1979.
4. Kaplan HS. Hodgkin's disease: unfolding concepts concerning its nature, management and prognosis. Cancer 45:2439–2474, 1980.
5. Pedersen-Bjergaard J, Nissen NI, Sörensen HM, et al. Acute non-lymphocytic leukemia in patients with ovarian carcinoma following long-term treatment with treosulfan (= dihydroxybusulfan). Cancer 45:19–29, 1980.
6. Brøgger A. Chromosome damage in human mitotic cells after in vivo and in vitro exposure to mutagens. *In* Genetic Damage in Man Caused by Environmental Agents (Berg K, ed). New York, NY, Academic Press, 1979, pp 87–99.
7. Wolff S. Sister chromatid exchange: the most sensitive mammalian system for determining the effects of mutagenic carcinogens. *In* Genetic Damage in Man Caused by Environmental Agents (Berg K, ed). New York, NY, Academic Press, 1979, pp 229–246.
8. Kurvink K, Bloomfield CD, Keenan KM, et al. Sister chromatid exchange in lymphocytes from patients with malignant lymphoma. Hum Genet 44:137–144, 1978.
9. Lambert B, Ringborg U, Harper E, et al. Sister chromatid exchanges in lymphocyte cultures of patients receiving chemotherapy for malignant disorders. Cancer Treat Rep 62:1413–1419, 1978.
10. Nevstad NP. Sister chromatid exchanges and chromosomal aberrations induced in human lymphocytes by the cytostatic drug adriamycin in vivo and in vitro. Mutat Res 57:253–258, 1978.
11. Raposa T. Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukaemic patients under cytostatic therapy. Mutat Res 57:241–251, 1978.
12. Gebhart E, Lösing J, Windolph B, et al. (Abstr) Chromosome and SCE studies in patients with cytostatic interval therapy. Ninth Annual Meeting of the European Environmental Mutagen Society, 1979. Mutat Res 74:193, 1980.
13. Schinzel A, and Schmid W. Lymphocyte chromosome studies in humans exposed to chemical mutagens. The validity of the method in 67 patients under cytostatic therapy. Mutat Res 40:139–166, 1976.
14. Hollander M, andWolfe DA. Nonparametric Statistical Methods. London, England, John Wiley & Sons, 1973.
15. Ardito G, Lamberti L, Ansaldi E, et al. Sister-chromatid exchanges in cigarette-smoking human females and their newborns. Mutat Res 78:209–212, 1980.
16. Falck K, Grohn P, Sorsa M, et al. Mutagenicity of urine of nurses handling cytostatic drugs. Lancet I:1250–1251, 1979.
17. Norppa H, Sorsa M, Vainio H, et al. Increased sister chromatid exchange frequencies in lymphocytes of nurses handling cytostatic drugs. Scand J Work Environ Health 6:299–301, 1980.
18. Brøgger A, and Waksvik H. Further evidence that the chromatid gap is a folding defect. Hereditas 89:131–132, 1978.
19. Gebhart E. Experimentelle Beiträge zum Problem der lokalen Achromasien (Gaps). Hum Genet 13:98–107, 1971.
20. Brøgger A. Caffeine-induced enhancement of chromosome damage in human lymphocytes treated wtih methyl-methane-sulphonate, mitomycin C and X-rays. Mutat Res 23:353–360, 1974.
21. Kusyk CJ, and Hsu TC. Mitotic anomalies induced by three inhalation halogenated anesthetics. Environ Res 12:366–370, 1976.
22. Grant CJ, Powell JN, and Radford SG. The induction of chromosomal abnormalities by inhalational anaesthetics. Mutat Res 46:177–184, 1977.
23. Evans HJ. Chromosome aberrations induced by ionizing radiations. Int Rev Cytol 13:221–321, 1962.
24. H :sum B, and Wulf HC. Sister chromatid exchanges in lymphocytes in operating room personnel. Acta Anaesthesiol Scand 24:22–24, 1980.
25. White AE, Takehisa S, Eger EI, et al. Sister chromatid exchanges induced by inhaled anesthetics. Anesthesiology 50:426–430, 1979.
26. Obe G, and Herha J. Chromosomal aberrations in heavy smokers. Hum Genet 41:259–263, 1978.
27. Lambert B, Lindblad A, Nordenskjöld M, et al. Increased frequency of sister chromatid exchanges in cigarette smokers. Hereditas 88:147–149, 1978.

Treatment of Recurrent Lymphomas With Vincristine, BCNU, Doxorubicin, and Prednisone (VBAP): A Southwest Oncology Group Study[1,2]

J. K. Weick,[3] S. E. Jones,[4] A. Haut,[5] R. Fisher,[6] and D. Dixon[6,*]

SUMMARY

Chemotherapy with vincristine, BCNU, doxorubicin, and prednisone (VBAP) was administered to 75 patients with malignant lymphoma, including both Hodgkin's disease and non-Hodgkin's lymphoma. All patients had prior chemotherapy with or without radiation therapy. Remissions were achieved in 41% of all patients, with only minor toxicity. The median duration of remission for Hodgkin's disease and non-Hodgkin's lymphoma was 51 and 22 weeks, respectively. The median survival for all patients was 34 weeks. The VBAP program is effective palliative chemotherapy for advanced malignant lymphoma.

[Cancer Treat Rep 65:611–614, 1981]

With the introduction of effective combination chemotherapy programs, the prognosis of all malignant lymphomas has been dramatically altered. It is to be expected that fully 80% of persons with Hodgkin's disease (HD) and between 50% and 80% of patients with non-Hodgkin's lymphoma (NHL) will attain complete and long-term remissions (1,2). However, this leaves 20% of all persons with advanced HD who do not achieve a complete remission and > 50% who subsequently relapse; in NHL this figure for patients with nodular histology is close to 100%, and for those with diffuse histologies it is between 50% and 60%. In an attempt to find a successful reinduction program for persons failing primary therapy, the Southwest Oncology Group (SWOG) has studied the combination of vincristine, BCNU, doxorubicin, and prednisone (VBAP) for persons with recurrent or relapsing HD or NHL.

PATIENTS AND METHODS

Any patients without known heart disease whose lymphomas recurred after primary induction chemotherapy and who had measurable disease were eligible for the study, provided they had received a cumulative total of < 390 mg/m² of doxorubicin. Prior nitrosourea therapy was similarly allowed. The usual clinical assessment was made of the extent of disease. Pathologic material was reviewed and classified according to the Rappaport classification for NHL and the Lukes-Butler classification for HD (3,4).

The treatment program entailed the use of four drugs as follows: in a 21-day cycle, vincristine was given at a total dose of 1 mg iv on Day 1, BCNU and doxorubicin were both given iv on Day 1 at a dose of 30 mg/m², and prednisone was given orally on Days 1–4 at a dose of 60 mg/m². Treatment was continued at 3-week intervals until a maximum dose of 550 mg/m² of doxorubicin was attained or until there was progressive disease; the study terminated with either end point. When the maximum dose of doxorubicin was reached, all drugs were

[1] Received Jan 25, 1980; revised Oct 24, 1980; accepted Nov 13, 1980.

[2] Supported in part by Public Health Service grants CA-04919, CA-13612, CA-13238, CA-03389, CA-20319, CA-03096, CA-21116, CA-22411, CA-04915, CA-12644, CA-16385, CA-12213, CA-16957, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Cleveland Clinic, OH.

[4] University of Arizona, Tucson.

[5] University of Arkansas, Little Rock.

[6] Southwest Oncology Group, Statistical Office, Kansas City, KS.

**Reprint requests to:* Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, KS 66103.

TABLE 1.—Response rate by histologic type in 69 evaluable patients

Disease	No. of patients treated	No. of PRs	No. of CRs	No. of PRs + CRs
HD	16	6 (37%)	3 (19%)	9 (56%)
Nodular lymphoma	25	10 (40%)	1 (4%)	11 (44%)
Diffuse lymphoma	33	9 (27%)	1 (3%)	10 (30%)
Mycosis fungoides	1	—	1	1
Totals	75	25 (33%)	6 (9%)	31 (41%)

discontinued and the patient was followed for relapse while off all chemotherapy. Dose modifications were made for pre-existing granulocytopenia, liver dysfunction, and persistent azotemia.

A complete remission (CR) was defined as the disappearance of all clinical evidence of tumor for a minimum of 4 weeks with no simultaneous appearance of new lesions. A partial remission (PR) was defined as a $\geq$ 50% decrease in the sum of the products of the maximal perpendicular diameters of all measured lesions. Survival and remission duration curves were calculated by the method of Kaplan and Meier (5), and comparisons of survival and remission duration curves between various groups of patients were made by the modified Wilcoxon test (6).

RESULTS

Seventy-five patients (28 women and 47 men) ranging from 20 to 74 years of age were entered in this trial (SWOG #7707). Sixteen patients had HD and 59 had various types of NHL. Sixty-nine patients were fully evaluable. Six persons did not complete two courses of therapy and were considered only partially evaluable. This includes four patients with early deaths from progressive disease after one course of medication, one lost to followup, and one who refused further treatment after one course of therapy. Objective responses were seen in 31 patients (41%) with six CRs (9%) and 25 PRs (33%). The breakdown of these responses by histologic type of lymphoma is given in table 1. More than half of the patients with HD responded and three achieved a CR. The patients with nodular lymphoma responded more often than those with diffuse lymphoma (44% vs 30%). One CR was observed among four patients with nodular mixed histology, and ten PRs occurred among 21 patients with nodular lymphocytic histology. Patients with diffuse histiocytic lymphoma were less likely to obtain a remission (three of 18 patients) compared with those with diffuse lymphocytic well-differentiated (three of five) or diffuse poorly differentiated (four of ten) lymphoma. One patient with mycosis fungoides experienced a CR. None of the 75 patients had previously received a nitrosourea. Thirty-one patients had previously received doxorubicin, all persons had previously received steroid therapy, and 74 of 75 had previously received vincristine therapy. Patients who had previously received doxorubicin were less likely to show a response (ten of 31 patients) than those who had not previously received doxorubicin (21 of 29; $P < 0.02$).

The duration of response for the entire group varied from 4 to 80 weeks, with a median of 22 weeks in the NHL group and 51 weeks in the HD group ($P = 0.09$) (fig 1). The median survival of all patients was 34 weeks. Responders survived a median of 59 weeks compared to 10 weeks for nonresponders ($P = 0.002$).

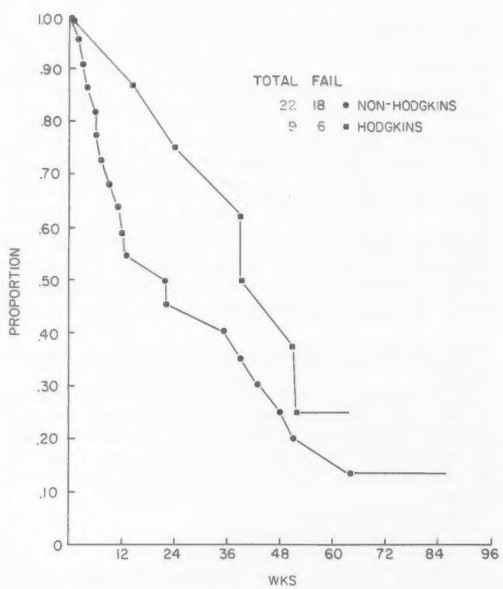

FIGURE 1.—Response duration among patients with HD or NHL.

TABLE 2.—Frequency of side effects associated with VBAP chemotherapy in 75 patients

Degree of toxicity	No. of patients with— Low wbc counts*	Low platelet counts†	Gastro-intestinal toxicity	Neuropathy	Cardiac toxicity
Mild	6 (8%)	1 (1%)	9 (12%)	1 (1%)	0
Moderate	7 (9%)	1 (1%)	7 (9%)	0	0
Severe	1 (1%)	2 (3%)	3 (4%)	0	0
Life-threatening	1 (1%)	0	0	0	0
Fatal	0	0	0	0	0
Totals	15 (20%)	4 (5%)	19 (25%)	1 (1%)	0

*Mild = 3000–3999 cells/mm^3; moderate = 2000–2999 cells/mm^3; severe = 1000–1999 cells/mm^3; life-threatening = < 1000 cells/mm^3.

†Mild = 75,000–99,999 cells/mm^3; moderate = 50,000–74,999 cells/mm^3; severe = 25,000–49,999 cells/mm^3; life-threatening = < 25,000 cells/mm^3.

VBAP was well-tolerated by most patients. The frequency of various side effects and their degrees of severity are listed in table 2. In particular, myelosuppression was quite mild despite heavy prior chemotherapy in most patients and impaired bone marrow reserves in many patients at the onset. No cardiomyopathy was observed.

DISCUSSION

As single agents, the vinca alkaloids, nitrosoureas, doxorubicin, and prednisone have established efficacy in previously treated patients. These response rates generally are about 20%–40%, with an average response duration of 20–24 weeks (7–11). It is thus difficult to evaluate the contribution of the combined program as compared with the individual agents. The ten responding patients of the 31 who previously had had doxorubicin, vincristine, and prednisone presumably are those "true" responders to BCNU. These responses were short in duration (median, 5 months) and included no CRs. Such numbers are similar to those previously cited for nitrosoureas alone. If, however, a similar comparison is made for the 29 patients not receiving prior doxorubicin, the number of responders (21 patients; 72%) surpasses values for doxorubicin as a single agent, suggesting additive benefit of combination therapy. The median survivals in the group as a whole surpassed those for the individual agents, making it likely that the combination of VBAP does have efficacy beyond that of the individual components.

In most cases, patients were treated with VBAP when relapse occurred during active treatment. Disease-free intervals ranging from 3 to 12 months were seen in eight of 31 responders and in five of the 44 nonresponders. This coincides with the generally accepted tenet that persons having a disease-free interval requiring no therapy may respond again to a subsequent treatment program.

Since every patient in this study had received extensive prior chemotherapy, the overall response rate of 41% is encouraging, especially in view of the mild toxic effects observed with this regimen. Severe toxicity was characteristic of earlier phase II trials with both nitrosoureas and doxorubicin. This has been obviated by the lower dose of medication without an apparent loss of efficacy. The response duration was usually between 5 and 12 months with excellent overall disease control.

REFERENCES

1. DEVITA VT, JR, SERPICK AA, and CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881–895, 1970.
2. JONES SE, GROZEA PN, METZ EN, ET AL. Superiority of adriamycin-containing combination chemotherapy in the treatment of diffuse lymphoma. Cancer 43:417–425, 1979.
3. RAPPAPORT H, WINTER WJ, and HICKS EB. Follicular lymphoma: a re-evaluation of its position in the scheme of malignant lymphoma, based on a survey of 253 cases. Cancer 9:792–821, 1956.
4. LUKES RJ, and BUTLER JJ. The pathology and nomenclature of Hodgkin's disease. Cancer Res 26:1063–1081, 1966.
5. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
6. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–223, 1965.
7. LESSNER HE. BCNU (1.3,bis(B-chloroethyl)–I-nitrosourea: Effects on advanced Hodgkin's disease and other neoplasia. Cancer 22:451–456, 1968.
8. REGE VB, and OWENS AH, JR. BCNU (NSC–409962) in the treatment of advanced Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. Cancer Chemother Rep 58:383–392, 1974.
9. WASSERMAN TH, SLAVIK M, and CARTER SK. Clinical comparison of the nitrosoureas. Cancer 36:1258–1268, 1975.

10. Coltman CA, Jr. Adriamycin (NSC 123127) in the treatment of lymphomas: Southwest Oncology Group studies. Cancer Chemother Rep 6:375–380, 1975.

11. Livingston RB, and Carter SK. Single Agents in Cancer Chemotherapy. New York, NY, IFI/Plenum, 1970, pp 298–337.

Iv Hyperalimentation and Chemotherapy for Stage III Testicular Cancer: A Randomized Study[1,2]

Melvin L. Samuels,* Debra E. Selig, Sheryl Ogden, Cynthia Grant, and Barry Brown[3,4]

SUMMARY

Thirty evaluable patients with stage III metastatic testicular cancer were prospectively randomized to receive iv hyperalimentation (IVH) starting on Day 1 of hospitalization. There were 16 IVH patients and 14 control patients and a total of 88 evaluable trials. The IVH patients received 35 kcals/kg/day of 25% dextrose with 4.25% amino acids, while control patients received 3 liters/day of 5% dextrose in 0.5 normal saline when gastrointestinal side effects from chemotherapy impaired the normal ingestion of food and fluids, usually by treatment Day 3 or 4. Chemotherapy was started on hospital Day 2 and consisted of vinblastine plus bleomycin, with a second randomization to low-dose cisplatin (75 mg/m^2) with the first and third courses. Four courses were programmed for each patient. IVH successfully blunted weight loss from the gastrointestinal side effects of chemotherapy. The control patients showed a mean weight loss of 4.89 kg/trial compared to 1.67 kg/trial for the IVH patients ($P < 0.001$). However, other serious side effects were not lessened. Granulocytopenia was severe in both groups. The mean leukopenia nadir, nadir day, and recovery day also showed no significant clinical differences. The control group developed two cases of bacteriologically proven septicemia, with prompt recovery in both cases, while the IVH group developed five cases of bacteriologically proven septicemia and two of pneumonia. The one death from infection was due to *Klebsiella pneumoniae* pneumonia, which appeared on treatment Day 4 and produced death on Day 8. The increased number of life-threatening infections in the IVH group was significant ($P = 0.047$). Only one case of septicemia was catheter-related. Seventy-two percent of the IVH patients were hyperglycemic during the early part of the trials and this may have been an important etiologic factor. The mean duration of IVH was 48 days for noninfected patients and 18 days for infected patients. The complete response rate for the IVH patients was 63% while the complete response rate was 79% ($P = 0.29$) for the control patients. Likewise, the addition of low-dose cisplatin did not significantly improve the initial complete response rate. With the addition of surgery for residual disease and secondary salvage chemotherapy, the complete response and probable cure rates are > 75% for the entire population. During the study, a bowel perforation from tumor occurred in an IVH patient with liver metastases

[1]Received May 1, 1980; revised Oct 21, 1980; accepted Nov 20, 1980.

[2]Supported by contract N01-CP-65866 from the Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine (*M. L. Samuels* and *C. Grant*), Nutrition and Food Service (*D. E. Selig*), Nursing (*S. Ogden*), and Biomathematics (*B. Brown*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]We thank Carol Hahn and Alma Swisher for the preparation of this manuscript.

Reprint requests to: Melvin L. Samuels, MD, Section of General Medicine and Clinical Oncology, Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

and we were able to continue chemotherapy and induce a complete remission that continues beyond 2 years. In addition, three control patients who had high weight loss after initial chemotherapy were changed to IVH, and two have remained in stable complete remission beyond the 2-year risk period. Aggressive effective chemotherapy was made possible by the addition of IVH in these select patients. However, in the patients with testis cancer receiving vinblastine, bleomycin, and cisplatin, IVH should be restricted to those who present with a significant weight loss or who fail to regain lost weight during rest periods between courses.

[Cancer Treat Rep 65:615–627, 1981]

Metastatic germinal neoplasia of the testis shows an approximate complete response (CR) rate of 70% using a combination of vinblastine, bleomycin, and cisplatin, but this chemotherapy program produces a substantial cachectogenic effect (1,2). Marked weight loss is caused by the gastrointestinal side effects of the chemotherapy, which include stomatitis, nausea, vomiting, and paralytic ileus (1,2). In previous reports of nonrandomized trials with vinblastine and bleomycin, Samuels et al (3) noted a median weight loss of 4.95 kg/trial in 24 trials without iv hyperalimentation (IVH), as opposed to a median weight gain of 0.45 kg in 15 IVH-supported trials. Furthermore, animal studies have shown that response to chemotherapy can be improved and tolerance to chemotherapy can be increased by concomitant IVH (4–6). Limited nonrandomized studies with cancer patients have produced similar results (6–8). Therefore, a prospective randomized trial was initiated to test the effects of IVH on response as well as on the severe side effects of the vinblastine and bleomycin program. A second randomization arm with cisplatin was created since it has been reported to increase the response to as well as the gastrointestinal toxicity of vinblastine and bleomycin (1,2,9) and since there have been no randomized trials reported comparing vinblastine and bleomycin and vinblastine, bleomycin, and cisplatin.

MATERIALS AND METHODS

Thirty-five patients were entered in the study between March 27, 1977, and September 4, 1978. They were stratified into three nutritional status categories: well-nourished ($\leq$ 6% weight loss); moderately malnourished ($>$ 6% but $<$ 12% weight loss); and severely malnourished ($>$ 12% weight loss). These weight loss categories were based on previous observations by Lanzotti et al (8), and not the criteria of 10% loss of usual body weight (10,11). Signed informed consent was obtained from patients, and block randomization for patient group assignment was done using a random numbers table and a system of sealed envelopes. Severely malnourished patients were not randomized but assigned to receive IVH. Each liter of hyperalimentation solution contained 25% glucose and 4.25% amino acids as Freamine II and supplied approximately 1000 calories. Supplementary vitamins, electrolytes, and trace elements were added to each liter as shown in table 1. IVH was begun the first day of each hospitalization and was continued throughout the course of the chemotherapy, terminating 24 hours before dismissal. Administration was by way of a percutaneous infraclavicular subclavian catheter, which was placed and maintained by the IVH team of the General Surgery Service. The aim was to supply a minimum of 35 kcals/kg/day. One liter was delivered over the first 24 hours, 2 liters was delivered over the second 24 hours, and $\geq$ 3 liters was delivered for the remainder of the hospitalization. Laboratory monitoring of the IVH included determination of serum sodium, potassium, chlorine, carbon dioxide, glucose, and creatinine each Monday, Wednesday, and Friday, and urine levels of sugar and acetone four times daily. If glycosuria was encountered in a patient with significantly elevated blood sugar concentrations, exogenous insulin was supplied or the glucose delivery rate was reduced. Determination of serum magnesium was performed weekly, as were SMA-12 multichannel chemistry studies. Control patients who developed significant gastrointestinal toxic effects received 3 liters of parenteral fluids daily, usually containing 5% glucose, 0.5 normal saline, and 40 mEq of potassium chloride. In the event of $>$ 12% weight loss after chemotherapy, control patients were crossed over to receive IVH at the discretion of the investigator.

Tumor presentation was classified as low volume (minimal disease) and large volume (advanced disease) according to previously described criteria (3). Response has been shown to be directly proportional to tumor volume (1,3).

TABLE 1.—Formulation of hyperalimentation solution

Substance*	Form provided	Amount	Vol (ml)	kcals
Protein equivalent	Freamine, 8.5%	39 g	500	156
Dextrose	Dextrose, 50%	250 g	500	850
Potassium	Potassium chloride, 2 mEq/ml	20 mEq	10	
Phosphate	Potassium phosphate, 6 mEq/ml	30 mEq	5	
Calcium	Calcium gluconate	1 g	10	
Sodium chloride	Sodium chloride, 2.5 mEq/ml	50 mEq	20	
Magnesium	Magnesium sulfate, 4 mEq/ml	10 mEq	2.5	
Multivitamins	MVI	5 ml		

*Phosphate, calcium, and multivitamins were added to only 1 liter of IVH solution every 24 hrs. Trace elements were added to 1 liter of IVH solution every 24 hrs as follows: zinc (as sulfate), 2.0 mg; copper (as sulfate), 1.0 mg; iodide (as potassium salt), 0.056 mg; manganese (as sulfate), 0.4 mg; and chromium (as chloride), 0.1 mg. Every 1000 ml contained 6.24 g of nitrogen and approximately 1000 kcals.

The chemotherapy program consisted of vinblastine and bleomycin, with one half of the study group randomized to receive sequential cisplatin as well. Four courses were programmed for each patient. The vinblastine dose was determined by risk category. Poor-risk patients were defined as those who had received major radiotherapy to the pelvis and para-aortic areas (tumor dose, 3500 rads) or who were > 40 years of age. Past experience has shown that patients with testis cancer who are > 40 years of age tolerate vinblastine and bleomycin chemotherapy poorly (3). All other patients were considered to be in the good-risk category. Weight loss was not a criterion for risk-group categorization. All good-risk patients received 0.5–0.6 mg/kg of vinblastine in two equal fractions given by iv push on Days 1 and 2, 24 hours after IVH was started. Poor-risk patients received 0.4–0.45 mg/kg of vinblastine. Bleomycin was given in a fixed dose of 30 units over 24 hours by continuous infusion for 5 consecutive days, starting on Day 2. Patients who were randomized to receive cisplatin received a dose of 75 mg/m^2 following the first and third courses of vinblastine and bleomycin. Cisplatin was administered in 200 ml of normal saline over a 2-hour period, after a pulse dose of 12.5 g of mannitol. Mannitol diuresis followed the cisplatin. Four liters of the mannitol solution (40 g of mannitol/liter of 5% glucose in 0.25 normal saline) was given at a rate of 200 ml/hour. During the period of cisplatin administration and diuresis, IVH was decreased to 1 liter every 24 hours. Cisplatin was administered after hematologic recovery, usually on Day 18 or 19. A 25% decrease in the dose of vinblastine was necessary in the event of severe hemorrhagic stomatitis, severe paralytic ileus, or bacteriologically proven sepsis.

Chemotherapy-monitoring procedures included a cbc each Monday, Wednesday, and Friday, pulmonary function studies and a chest roentgenogram before each admission, and a creatinine clearance test before each course of cisplatin.

Statistical comparisons of data from the IVH and control groups were made by means of Fisher's exact test and Student's t test. Survival curves were analyzed by the Wilcoxon method.

RESULTS

Table 2 summarizes the randomization of patients into the three weight loss categories and four treatment programs. Eight (23%) of the 35 randomized patients presented with significant weight loss but were fully ambulatory. Two of the eight weight loss patients showed > 12% weight loss compared to their usual weight and were assigned to receive IVH. There were three early deaths. One IVH patient died on Day 8 from a fulminant bilateral pneumonia caused by *Klebsiella pneumoniae*. A second patient, in the control group, died on Day 10 from aspiration pneumonia. A third patient, in the IVH group, developed acute tubular necrosis with oli-

TABLE 2.—Randomization of patients according to weight loss

Program	Total No. of patients	No. of patients with weight loss of —		
		0–6%	> 6%–12%	> 12%
Vinblastine and bleomycin (control)	8	7	1	—
Vinblastine, bleomycin, and cisplatin (control)	7	6	1	—
Vinblastine and bleomycin plus IVH	10	6	2	2
Vinblastine, bleomycin, and cisplatin plus IVH	10	8	2	—
Totals	35	27	6	2

guric renal failure on Day 8 and was transferred to another hospital for hemodialysis; he died on Day 24 from an accidental air embolus while receiving dialysis. Although these three patients received one complete course of therapy, they were excluded from response and survival data because of their early deaths. Likewise, toxicity data become difficult to evaluate in these three patients since nadir and recovery times are unavailable. Thus, 30 randomized patients constitute the evaluable study population.

Since each patient was scheduled to receive four courses of therapy, a total of 120 courses should have been administered. However, only 88 courses were evaluable. The reasons for the unevaluability of 32 courses are summarized in table 3. Three control patients were crossed over to receive IVH, for a total of five nonevaluable courses. These patients had demonstrated further weight loss of > 12% following treatment and parenteral nutritional supplementation was required. Four IVH patients developed uncontrolled hyperglycemia in their first course of therapy, and IVH was not reinstituted in subsequent courses.

Table 4 compares the number of minimal disease and advanced disease presentations in the IVH and control groups, since randomization was not done

TABLE 3.—Program changes producing unevaluable trials

Program	No. of patients	No. of evaluable trials	No. of patients requiring IVH randomization change	Reasons for unevaluable trials	Total No. of unevaluable trials
Vinblastine and bleomycin (control)	7	24	1	> 12% wt loss (1);* neuropathy (2); refused further treatment (1)	4
Vinblastine, bleomycin, and cisplatin (control)	7	24	2	> 12% wt loss (4)	4
Vinblastine and bleomycin plus IVH	6	19	3	Sepsis (4); program change† (1)	5
Vinblastine, bleomycin, and cisplatin plus IVH	10	21	6	Uncontrolled hyperglycemia (10); uncontrolled hyperglycemia with sepsis (2); program change† (6); poor tumor response, surgical debulking (1)	19

*Nos. in parentheses = No. of trials.
†Salvage chemotherapy because of poor tumor response.

TABLE 4.—Presentations of randomized patients

Presentation	No. of IVH patients	No. of control patients
Biomarker elevation with gynecomastia and galactorrhea	2	0
Minimal pulmonary disease	1	2
Advanced pulmonary disease	8	7
Advanced abdominal disease		
Liver metastasis	2	1
Hydronephrosis	0	2
Perforation of gastrointestinal tract	1	0
Palpable tumor	2	2
Totals	16	14

to assure even distribution of advanced presentations. There were five patients with minimal presentations, three in the IVH group and two in the control group. The distribution of the 25 patients with advanced presentations was 13 in the IVH group and 12 in the control group. Table 5 gives the distribution by the major histologic tumor types: embryonal carcinoma, teratocarcinoma, and choriocarcinoma. Choriocarcinoma is considered to be the most resistant (1,3). Although there were three patients with choriocarcinoma in the IVH group and one in the control group, the difference was not significant ($P = 0.35$). Likewise, the distribution of patients with embryonal carcinoma and teratocarcinoma showed no significant difference.

Response data are summarized for the IVH and control groups in table 6. The CR rates for the initial chemotherapy program are 63% (ten of 16 patients) for the IVH group and 79% (11 of 14 patients) for the control group. The difference is not significant ($P = 0.29$). Table 6 summarizes results with surgery for residual disease and with secondary chemotherapy that was initiated for patients with partial remissions (PR) that appeared stable or those with CRs that had relapsed. The total impact of primary chemotherapy, surgery for residual disease, and secondary salvage chemotherapy is shown in the

TABLE 5.—Histologic types in 30 randomized patients

Histologic type	No. of IVH patients	No. of control patients
Embryonal carcinoma	7	6
Teratocarcinoma	6	7
Choriocarcinoma	3	1
Totals	16	14

TABLE 6.—Response of IVH and control patients

Variable	No. of IVH patients	No. of control patients
Randomized patients, total	16	14
CR	10 (63%)	11 (79%)
PR	4	2
Disease-free following surgery	3	1
PR induced to stable CR with secondary program	1	1
Relapse from CR	3* (2 dead)	2 (2 dead)
No evidence of disease	12 (75%)	11 (79%)

*Remaining patient was reinduced to CR with a secondary program.

rates for no evidence of disease. The overall salvage rates in the IVH and control groups were 75% and 79%, respectively. CR was also compared in the two chemotherapy groups and again showed no significant difference. A CR rate of 75% was achieved with the three-drug program and a CR rate of 69% was achieved with the vinblastine and bleomycin program. However, there was an unequal distribution of patients with embryonal carcinoma, with nine patients in the cisplatin group and four in the vinblastine and bleomycin group. Patients with teratocarcinoma and choriocarcinoma were equally distributed between the two groups.

Response was further analyzed according to risk-group status. Eight patients received low-dose vinblastine and five of these showed stable CRs. Twenty-two patients received high-dose vinblastine and 16 achieved a CR. A comparison of the proportions of CRs by risk group does not show a significant difference ($P = 0.5$). However, the poor-risk group had the advantages of only one abdominal presentation, no cases of choriocarcinoma, and three of the five minimal disease presentations. These variables all favorably influence response (3).

The 30 evaluable patients plus the two patients with high weight losses were analyzed to determine the effect on response of weight loss at presentation. Two patients presented with > 12% weight loss. A CR of 4 months' duration was achieved in one patient who subsequently was reinduced to stable CR with a salvage chemotherapy program. The remaining patient with a high weight loss presented with multiple large lung metastases and achieved a striking PR (90%); however, the patient died during the fourth chemotherapy course from brain metastases. Five evaluable patients presented with > 6%

TABLE 7.—Effect of IVH on wt change

Program	No. of courses	Mean wt change (kg) 1st course	2nd course	3rd course	4th course	Mean wt change/course (kg)	Mean wt change/course for control and IVH groups (kg)
Vinblastine and bleomycin (control)	24	–5.94	–3.06	–3.50	–3.89	–4.19 (±0.51)*	–4.89 (±0.384)
Vinblastine, bleomycin, and cisplatin (control)	24	–8.70	–4.52	–5.07	–3.31	–5.60 (±0.55)	
Vinblastine and bleomycin plus IVH	19	–0.57	–2.60	+0.48	–2.92	–1.23 (±0.73)	–1.67 (±0.84)
Vinblastine, bleomycin, and cisplatin plus IVH	21	–3.79	–2.77	+0.63	–2.95	–2.06 (±0.84)	

*Nos. in parentheses = SEM.

but < 12% weight loss. Two patients achieved CRs. Among the 25 remaining patients with < 6% weight loss, there were 19 CRs. The difference between the proportion of CRs in patients with > 6% weight loss and that in patients with < 6% weight loss is not statistically significant ($P = 0.11$). However, a trend toward an improved CR rate in the patients with low weight losses is evident.

The weight changes per treatment course were determined for the randomized patients and are summarized in table 7. The mean weight loss for the 48 control courses is 4.89 kg/trial compared to 1.67 kg/trial for the 40 IVH trials ($P < 0.001$). There was also a significant difference in weight loss between the vinblastine and bleomycin control and IVH groups, as well as among the vinblastine, bleomycin, and cisplatin control and IVH groups ($P < 0.001$).

Table 8 shows the levels of the secretory proteins albumin and transferrin before and after chemotherapy. The difference between the groups in the decrement produced by chemotherapy is not significant.

Abnormal results of liver function studies during IVH have been reported (12). Likewise, we have reported abnormal results of liver function studies following vinblastine and bleomycin chemotherapy (13). Table 9 compares serum alkaline phosphatase, SGOT, and serum bilirubin abnormalities in the control and IVH groups. Eleven IVH patients showed elevations of alkaline phosphatase, SGOT, or both, while four control patients showed almost identical mean elevations to approximately two times maximal normal values. These elevated values were seen at or near Day 12 and usually returned to normal by Weeks 3–4. IVH produces a significant increase in the number of trials showing transient liver function abnormalities.

Measurements of myelosuppression are shown in tables 10–13. Table 10 compares the leukopenia nadirs, nadir days, and recovery days for each course for the control and IVH groups. There is no significant clinical difference in any of these parameters. Table 11 shows the degrees of granulocyte depression for the control and IVH groups. The number of trials showing < 500 granulocytes/mm³ was not

TABLE 8.—Effect of IVH on serum albumin and serum transferrin

	IVH courses	Control courses	P value
Albumin (g/100 ml)			
Base value	4.00 (±0.74)*	4.01 (±0.58)	0.5
Following chemotherapy	3.79 (±0.74)	3.76 (±0.56)	
Transferrin (mg/100 ml)			
Base value	224.90 (±6.02)	206.50 (±6.90)	0.3
Following chemotherapy	204.90 (±6.93)	174.10 (±7.23)	

*Values = mean (± SEM) pretreatment levels and nadirs for all IVH and control courses. Difference in decrements produced by chemotherapy is not significant between groups.

TABLE 9.—Effect of IVH on liver function

	Control courses	IVH courses	*P* value
Total No. of evaluable courses	48	40	
Alkaline phosphatase elevation			
No. of courses	8	14	0.04
Mean milliunits/ml ± SEM	150.37 ± 19.58	155.85 ± 13.80	
SGOT elevation			
No. of courses	5	12	0.02
Mean milliunits/ml ± SEM	62.40 ± 2.60	75.25 ± 9.40	
Bilirubin elevation			
No. of courses	3	4	0.25
Mean mg/100 ml ± SEM	1.33 ± 0.13	1.38 ± 0.08	

TABLE 10.—Leukopenia in control and IVH patients

	Control	IVH
No. of evaluable courses	48	40*
1st course		
No. of evaluable trials	14	15
Mean nadir ± SEM (cells × 10^3/mm^3)	0.87 ± 0.12	0.88 ± 0.20
Mean nadir day	8	9
Mean recovery day†	14	14
2nd course		
No. of evaluable trials	14	10
Mean nadir ± SEM (cells × 10^3/mm^3)	0.78 ± 0.14	0.68 ± 0.09
Mean nadir day	9	8
Mean recovery day	17	15
3rd course		
No. of evaluable trials	11	7
Mean nadir ± SEM (cells × 10^3/mm^3)	0.85 ± 0.14	1.2 ± 0.30
Mean nadir day	8	8
Mean recovery day	16	16
4th course		
No. of evaluable trials	9	5
Mean nadir ± SEM (cells × 10^3/mm^3)	0.88 ± 0.24	1.05 ± 0.42
Mean nadir day	8	9
Mean recovery day	16	16

*There were 3 courses without leukopenia.
†Total leukocyte count was 2000/mm^3.

significantly less for the IVH group ($P = 0.24$). Table 12 compares the platelet depressions following chemotherapy in the IVH and control groups. In 66% of the IVH trials platelet counts were > 100,000/mm^3 compared to 33% of the control trials. Platelet count nadirs were < 50,000/mm^3 in only 2% of the IVH trials compared to 33% of the control trials ($P = 0.0001$). Thus, IVH trials were clearly less injurious to platelets than were control trials. The effects of the chemotherapy per se on platelet counts are summarized in table 13. The addition of cisplatin to the chemotherapy regimens did not produce a statistically significant difference in platelet depression ($P = 0.33$).

Septicemia and pneumonia were the life-threatening infections that complicated the treatment programs, and their distribution in the various groups is summarized in table 14. In the 48 control courses, two patients developed septicemia after chemotherapy and both recovered. Five patients developed septicemia in the 40 IVH courses and two more developed granulocytopenic pneumonia. One of the two patients with pneumonia died as a result of fulminant *K. pneumoniae*. This was one of the randomized patients unevaluable for response.

The difference between the number of life-threatening infections in the IVH and control groups is significant ($P = 0.047$). Four infections followed the

TABLE 11.—Granulocyte depression in control and IVH trials

Granulocyte count (cells/mm³)	No. of control trials	No. of IVH trials
2000–2999	0	1 (2%)
1000–1999	1 (2%)	2 (5%)
500–999	4 (8%)	3 (8%)
< 500	43 (90%)	34 (85%)
Totals	48	40

TABLE 12.—Platelet depression in control and IVH trials

Platelet count (cells/mm³)	No. of control trials	No. of IVH trials
>150,000	5 (10%)	11 (28%)
100,000–149,999	11 (23%)	15 (38%)
50,000–99,999	16 (33%)	13 (32%)
25,000–49,999	15 (31%)	1 (2%)
< 25,000	1 (2%)	0
Totals	48	40

first course of chemotherapy, three followed the second, and two followed the third. Infection occurred in two of the eight patients with moderate and high weight losses. However, two additional infections occurred in patients who developed > 6% weight loss following chemotherapy. The remaining five infected patients were in the 6% weight loss group.

Only one case of septicemia was catheter-related. The infecting organism was *Staphylococcus epidermidis*, and there was an associated thrombosis of the subclavian and internal jugular veins. There were no other catheter-related infections. All catheter tips were cultured and all were negative except for that from the above-mentioned patient. Two other patients developed fever with signs of thrombophlebitis. In both patients the catheters were removed; the blood cultures and catheter tip cultures were negative. Temperature levels promptly returned to normal.

TABLE 13.—Platelet depression with vinblastine and bleomycin vs vinblastine, bleomycin, and cisplatin*

Platelet count (cells/mm³)	No. of vinblastine and bleomycin trials	No. of vinblastine, bleomycin, and cisplatin trials
> 150,000	10 (23%)	6 (13%)
100,000–149,999	10 (23%)	16 (36%)
50,000–99,999	16 (37%)	13 (29%)
25,000–49,999	7 (16%)	9 (20%)
< 25,000	0	1 (2%)
Totals	43	45

*Although the % of trials with patients showing serious platelet depressions (< 50,000 cells/mm³) appears to be greater in the cisplatin group, the difference is not statistically significant (P = 0.33).

The mean duration of IVH was 48 days for the noninfected patients and 18 days for the infected patients. The longest period of IVH was 113 days. This occurred in a patient who developed a perforation of the second portion of the duodenum from tumor infiltration, with massive hematochezia shortly after admission. This patient also had liver and lung metastases. He required radiographically directed occlusion of the gastroduodenal artery and 1000 rads of radiation to control hemorrhage, plus five courses of vinblastine, bleomycin, and cisplatin. He is in CR at 2 years from Day 1 of therapy.

Complicating septicemia was an early finding seen between Days 6 and 10. Our policy was to remove indwelling subclavian lines from patients with septicemia as soon as possible, provided an alternate venous pathway was available for intensive antibiotic therapy. The mean dose of vinblastine was 0.5 mg/kg for the trials of the two patients with pneumonia and the seven with septicemia, while the mean dose was 0.54 mg/kg for the 79 trials of patients with negative cultures.

Blood sugar levels were studied in the 16 patients who received 40 evaluable IVH courses. Patients in 29 of the 40 trials (72%) were hyperglycemic, with blood sugar levels > 170 mg/dl. Of the seven infected IVH patients, six had hyperglycemia, with a mean maximum blood sugar level of 303 mg/dl noted on Day 5. Noninfected patients in 23 of the remaining 33 IVH courses showed a mean maximum blood sugar level of 260 mg/dl on Day 7. Unfortunately, blood glucose determinations were done only weekly in the control patients, so a comparison with IVH patients is difficult. None of the controls demonstrated hyperglycemia, based on the limited observations.

In addition to the bacteriologically proven infections, there was a febrile response > 101°F in 42 courses during the granulocytopenia immediately following chemotherapy. A minimum of three closely spaced blood cultures were negative and there were no clinically detectable foci of infection. These courses with febrile granulocytopenia are segregated by control and IVH groups in table 15. Patients in 16 of 48 (33%) control trials were febrile, in comparison to patients in 26 of 40 (65%) IVH trials. The difference is highly significant (P = 0.003). The incidences of granulocytopenic fever by chemotherapy program are shown in table 16. Although the cisplatin group contained more trials with febrile patients, the difference is not significant (P = 0.10).

TABLE 14.—Septicemia and pneumonia in control and IVH trials*

Group	No. of patients	No. of trials	Pneumonia			Sepsis		
			No. of patients	Course No.	Organism	No. of patients	Course No.	Organism
Vinblastine and bleomycin (control)	7	24	0	—	—	0	—	—
Vinblastine, bleomycin, and cisplatin (control)	7	24	0	—	—	2	1	*Streptococcus* group C
							3	*Staphylococcus aureus*
Vinblastine and bleomycin plus IVH	6†	19†	1	1	*K. pneumoniae*	2	1	*Staphylococcus epidermidis*
							2	*Bacillus megaterium*
Vinblastine, bleomycin, and cisplatin plus IVH	10	21	1	2	None isolated	3	1	*Staphylococcus aureus*
							2	*Escherichia coli* and *Enterobacter aerogenes*
							3	*Staphylococcus epidermidis*

*There were 2 patients with life-threatening infections in 48 control trials vs 7 patients in 41 IVH trials ($P = 0.047$).
†Not counting 1 patient who was unevaluable because of early death.

TABLE 15.—Granulocytopenic fever in control and IVH trials

Program	No. of trials	No. of trials with fever > 101°F	No. of days with fever > 101°F	Mean No. of days with fever trial
Control	48	16 (33%)	55	3.4
IVH	40	26 (65%)	96	3.7

TABLE 16.—Granulocytopenic fever in vinblastine and bleomycin vs vinblastine, bleomycin, and cisplatin trials

Program	No. of trials	No. of trials with fever > 101°F	No. of days with fever > 101°F	Mean No. of days with fever/trial
Vinblastine and bleomycin	43	17 (39%)	66	3.5
Vinblastine, bleomycin, and cisplatin	45	25 (55%)	85	3.4

Patients who demonstrated febrile granulocytopenia were actively treated with broad-spectrum antibiotics.

Severe stomatitis that interfered with swallowing and required narcotics on a regular basis for pain relief occurred in 10% of the control trials and in 20% of the IVH trials (table 17). The difference is not significant ($P = 0.17$).

The survival curves for the IVH and control groups (fig 1) show no significant difference ($P < 0.5$, Wilcoxon two-tailed test). However, these survival curves give the total uncensored impact of the primary chemotherapy program, surgical extirpation of residual disease (pure teratoma in three patients and teratoma plus embryonal carcinoma in one), and secondary salvage programs.

TABLE 17.—Oral toxicity in IVH and control trials

Program	No. of evaluable courses	No. of courses with—		
		No toxicity	Mild toxicity	Severe toxicity
Control	48	13	30	5 (10%)
IVH	40	8	24	8 (20%)

DISCUSSION

As expected, IVH significantly blunted weight loss from chemotherapy but did not produce the weight gain observed in preliminary studies (3). Lowry and Brennan (12) have stated that 26 kcals/kg daily is adequate for cancer patients, although their population was elderly and inactive with advanced disease and showed a mean weight loss of 17.8% before therapy. However, Warnold et al (14) noted that both the daily energy expenditure and the mean resting metabolic rate were significantly greater in cancer patients than in control patients. They recommend 33.5 ± 2.1 kcals/kg daily, a figure we exceeded. The weight loss in the IVH patients may partially be explained by fever. Patients in 42 of the 88 trials had granulocytopenic fever > 101°F in the postchemotherapy period. Furthermore, patients in an additional seven trials had septicemia and two had pneumonia. Each Fahrenheit degree rise increases the energy expenditure by 7.2% (15), while a major infection may increase basal energy expenditure by 20%–50%.

The major side effect of the vinblastine and bleomycin chemotherapy program was myelosuppression, with severe leukopenia and granulocytopenia. The mean nadir appeared on approximately the 8th treatment day, with recovery at or near Day 16. There was no diminution in the degree of myelosuppression by IVH, and the mean nadir and recovery days remained unchanged. The addition of 75 mg/m² of cisplatin to the first and third courses did not significantly change the degree of leukocyte depression or the pattern of recovery. In a recently reported study of chemotherapy for lung cancer, Issell et al (16) reported that IVH decreased the degree of granulocytopenia but did not change the nadir day or recovery time. That program was much less myelosuppressive than the presently reported study program.

Schwartz et al (7) have reported that IVH strikingly reduced the gastrointestinal toxicity of high-

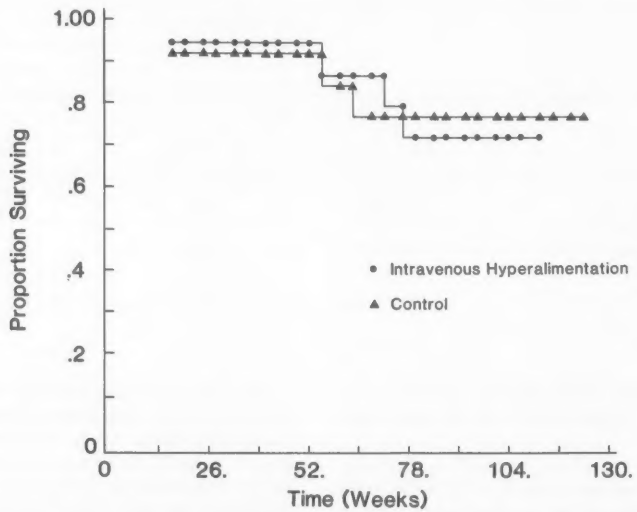

FIGURE 1.—Long-term survival and probable cure in patients with metastatic testicular cancer.

dose 5-FU in a small group of ten evaluable patients. Leukopenia (reportedly brief) was observed in only two patients. Although tolerance to high-dose 5-FU was improved, the response rate was not correspondingly altered. Souchon et al (6) noted improved tolerance to 5-FU in Sprague-Dawley rats receiving IVH but reported no hematologic data. Unfortunately, there is a paucity of literature addressing this point.

An unexpected finding of this study was the increased incidence of non-catheter-related serious infections in the IVH group. Catheter-related septicemia is likely to develop with some diseases, such as acute pancreatitis (17), in which a 17% incidence of septicemia has been recorded within the first 10 days of IVH. The infecting organisms consist predominantly of *Staphylococcus aureus* and *Staphylococcus epidermidis*. In a prospective study, Curry and Quie (18) noted septicemia in 13 of 49 IVH patients (27%), with *Candida albicans* the predominant infecting organism. The average duration of IVH was 26.4 days in patients in whom septicemia developed, as opposed to 10.3 days in patients without septicemia. However, Copeland et al (19) studied 93 cancer patients receiving long-term IVH and reported only three documented septicemias that were catheter-related. Two patients developed *C. albicans* septicemia, with one death. The remaining patient had *Streptococcus fecalis* septicemia. Copeland et al (19) associate the very low, 2.2% catheter-related clinical sepsis rate with the fact that a small number of specially trained personnel were responsible for the IVH program and that the IVH system was used only to deliver a nutritional solution.

We noted five cases of septicemia in 40 IVH trials, only one of which was associated with a culture-positive catheter tip, tending to confirm the observations of Copeland et al (19). There were no cases of fungal septicemia, even though the mean duration of IVH was 48 days. Of the seven IVH and control patients with septicemia, two were infected with *Staphylococcus aureus*, two with *Staphylococcus epidermidis*, one with *Streptococcus* group C, one with *B. megaterium*, and one with both *Escherichia coli* and *Enterobacter aerogenes*. Aside from the catheter-related *Staphylococcus epidermidis* infection, a possible focus was found for one *Staphylococcus aureus*-infected patient on IVH in whom the organism was cultured from the mouth when stomatitis was severe. The one *Streptococcus* group C infection was associated with a cellulitis of the cheek area (non-IVH patient). There were no deaths from septicemia and no patient sustained more than one major infection. Granulocytopenia was severe in both infected and noninfected patients, and there was no significant difference between IVH and control groups.

Two recent randomized studies have also shown an increase in infection in IVH patients. Abel et al (20) noted a 40% incidence of pneumonia and mediastinitis in cardiac patients randomized to postoperative parenteral nutrition, as opposed to an 8% incidence in controls. The death rate was increased to 20% in the IVH group versus 12.5% in the control group. Holter and Fischer (11) randomized 56 surgical carcinoma patients with weight losses > 10 pounds. The IVH patients received 3 days of preoperative parenteral nutrition followed by 10 days of additional IVH, while the control patients received the usual fluid and electrolyte replacement therapy. Eighty percent of the IVH patients showed an increase in weight, as opposed to 11% of controls. The serum albumin level showed a mean rise from 2.8 to 3.3 g/100 ml in the IVH group. The control group did not show a significant rise > 2.7 g/100 ml. However, there were two deaths from pneumonia in the IVH group (6.6%), with none in the control group.

Hyperglycemia was present in patients in 72% of our IVH trials, including six of seven IVH patients with life-threatening infections. Hyperglycemia could not be controlled in four patients in the vinblastine, bleomycin, and cisplatin plus IVH group and resulted in early discontinuation of IVH. Chemotherapy has been noted to produce catecholamine discharge and this could be important in the production of glucose intolerance (21,22). Porte et al (23) infused 6 μg/minute of epinephrine into normal volunteers and noted marked hyperglycemia. Immunoreactive insulin remained at basal levels until the infusion was discontinued. When epinephrine was used with an insulinogenic stimulus such as glucose or tolbutamide, the insulin levels were lower than with the stimulus alone. Therefore, epinephrine was believed to inhibit pancreatic insulin release.

Septicemia is a well-known cause of catecholamine excess, with increased blood levels of glucagon, cortisol, and glucose and inappropriately low insulin levels (24). However, most of the hyperglycemic patients in our study were not infected. Ryan (15) has noted a 15% incidence of hyperglycemia in 200 consecutive IVH patients and notes that patients who are predictably more vulnerable to hyperglycemia include those with renal or hepatic failure, pancreatitis, sepsis, burns, steroid therapy, immunosuppressant drug therapy, and diabetes. The concomitant administration of IVH with 25% dextrose and stressful chemotherapy markedly increases glucose intolerance and should be added to this list of risk factors.

Hyperglycemia may have been an important factor in the increased number of septic complications in the IVH group. Arieff and Carroll (25) noted that 27% of nonketotic hyperosmolar comas with hyperglycemia are apparently precipitated by infection but that an additional 30% of patients develop serious infections as a late complication, which frequently prove fatal. Goodgame and Fischer (17) reported that 25% of patients with acute pancreatitis receiving IVH became glucose-intolerant during the early phase of the disease. Septicemia as an early complication was increased to 17%. Van Oss and Border (26) have shown that intermittent brief episodes of hyperglycemia depress the in vitro phagocytic ingestion of opsonized gram-positive and gram-negative bacteria by polymorphonuclear leukocytes.

Liver function abnormalities were demonstrated to be significantly greater in the IVH group. Lowry and Brennan (12) recently reported increases above the normal range on two of three liver function tests in 33 of 40 malnourished cancer patients. These findings usually were observed on Day 14 from the start of parenteral feeding and the elevations were 1½–2 times normal. The functional significance of this finding is not known. Liver biopsy results have been variable, consisting of fatty infiltration, glycogen deposition, and cholestasis. Lowry and Brennan (12) consider an underlying steatosis secondary to IVH to be the most likely cause. Animal studies suggest that concentrated dextrose solutions are capable of producing fat or glycogen infiltration (27). Increased fatty acid synthesis within the liver can result from excessive carbohydrate intake (28).

We were unable to show an improvement in CR or survival in the IVH group. However, survival data are complicated by the addition of other therapies in patients achieving CR who relapsed or in patients who achieved only a partial response. Surgical extirpation of residual recalcitrant tumor foci is now an accepted part of the treatment for stage III disease. Secondary salvage programs are effective in producing further stable CRs.

Lanzotti et al (8) have shown that among patients with high weight losses only, the IVH group has a response rate of 50% to chemotherapy compared to no response in the non-IVH group. We were unable to confirm this finding, since only eight of our patients were in the moderate- or high-weight-loss category. Most young patients with testis cancer present with no significant weight loss. Our aim was to preserve normal protein-calorie nutrition throughout the treatment period and to improve patient tolerance to chemotherapy. Unfortunately, when treating advanced testicular cancer, the chemotherapy program must be aggressive, and in this particular setting the routine parenteral administration of 25% glucose as the primary calorie source may prove harmful. However, of the 32 patients in this study, two required IVH from the start because of high weight loss and three non-IVH patients were crossed over to IVH because of further severe weight loss from chemotherapy. One IVH patient would have died without IVH because of bowel perforation by tumor, and he is in CR. These six patients directly benefited from IVH, with four living and in CR. However, it seems clear from this study that IVH with a 25% glucose and 4.25% amino acid mixture should not be administered to the well-nourished patient with the aim of minimizing weight loss or decreasing the toxicity of chemotherapy. In testis cancer patients receiving vinblastine, bleomycin, and cisplatin, IVH should be restricted to those who present with a significant weight loss or who fail to regain lost weight during rest periods between courses.

REFERENCES

1. Samuels ML, Johnson DE, Brown B, et al. Velban plus continuous infusion bleomycin (VB-3) in stage III advanced testicular cancer. Results in 99 patients with a note on high-dose Velban and sequential *cis*-platinum. *In* Cancer of the Genitourinary Tract (Samuels ML, and Johnson DE, eds). New York, NY, Raven Press, 1979, p 159.
2. Einhorn LH, and Donohue JP. Chemotherapy for disseminated testicular cancer. Urol Clin North Am 4:407–423, 1977.
3. Samuels ML, Lanzotti VJ, Holoye PY, et al. Combination chemotherapy in germinal cell tumors. Cancer Treat Rev 3:185–204, 1976.
4. Steiger E, Oram-Smith J, Miller E, et al. Effects of nutrition on tumor growth and tolerance to chemotherapy. J Surg Res 18:445–461, 1975.
5. Cameron IL, and Rogers W. Total intravenous hyperalimentation and hydroxyurea chemotherapy in hepatoma-bearing rats. J Surg Res 23:278–288, 1977.
6. Souchon EA, Copeland EM, Watson P, et al. Intravenous hyperalimentation as an adjunct to cancer chemotherapy with 5-fluorouracil. J Surg Res 18:451–454, 1975.
7. Schwartz GF, Green HL, Bendon ML, et al. Combined parenteral hyperalimentation and chemotherapy in the treatment of disseminated solid tumors. Am J Surg 121:169–173, 1971.
8. Lanzotti VJ, Copeland EM, III, George SL, et al. Cancer chemotherapeutic response and intravenous hyperalimentation. Cancer Chemother Rep 59:437–439, 1975.
9. Stoter G, Sleijfer DT, Vendrick CPK, et al. Combination chemotherapy with *cis*-diammine-dichloro-platinum, vinblastine and bleomycin in advanced testicular non-seminoma. Lancet 1:941–945, 1979.
10. Copeland EM, MacFayden BV, Lanzotti VJ, et al. Intravenous hyperalimentation as an adjunct to cancer chemotherapy. Am J Surg 129:167–173, 1975.
11. Holter AR, and Fischer JE. The effects of perioperative hyperalimentation on complications in patients with carcinoma and weight loss. J Surg Res 23:31–34, 1977.
12. Lowry SF, and Brennan MF. Abnormal liver function during parenteral nutrition: relation to infusion excess. J Surg Res 26:300–307, 1979.

13. SAMUELS ML, JOHNSON DE, and HOLOYE PY. Continuous intravenous bleomycin (NSC-125066) therapy with vinblastine (NSC-49842) in stage III testicular neoplasia. Cancer Chemother Rep 59:563–576, 1975.
14. WARNOLD I, FALKHEDEN T, HULTEN T, ET AL. Energy intake and expenditure in selected groups of hospital patients. Am J Clin Nutr 31:742–749, 1978.
15. RYAN JA, JR. Complications of total parenteral nutrition. *In* Total Parenteral Nutrition (Fischer JE, ed). Boston, MA, Little, Brown, and Co, 1976, p 55.
16. ISSELL BF, VALDIVIESO M, ZAREN H, ET AL. Protection against chemotherapy toxicity by hyperalimentation. Cancer Treat Rep 62:1139–1143, 1978.
17. GOODGAME JT, and FISCHER JE. Parenteral nutrition in the treatment of acute pancreatitis. Effect on complications and mortality. Ann Surg 186:651–658, 1977.
18. CURRY CR, and QUIE PG. Fungal septicemia in patients receiving parenteral hyperalimentation. N Engl J Med 285:1221–1224, 1971.
19. COPELAND EM III, MACFAYDEN VB, MCGOWN C, ET AL. The use of hyperalimentation in patients with potential sepsis. Surg Gynecol Obstet 138:377–380, 1974.
20. ABEL RM, FISCHER JE, BUCKLEY MJ, ET AL. Malnutrition in cardiac surgical patients. Results of a prospective, randomized evaluation of early postoperative, parenteral nutrition. Arch Surg 111:45–50, 1976.
21. CREASY WA. Vinca alkaloids. *In* Cancer Chemotherapy III (The Forty-Sixth Hahnemann Symposium) (Brodsky I, Kahn SB, and Conroy JF, eds). New York, NY, Grune and Stratton, 1978, p 49.
22. HERMANN EH, and VICK JA. The acute pharmacological actions of daunomycin in the dog and monkey. Pharmacology 3:291–304, 1970.
23. PORTE D, GRABER AL, KUZUYA T, ET AL. The effect of epinephrine in immunoreactive insulin levels in man. J Clin Invest 45:228–236, 1966.
24. MARCHUK JB, FINLEY RJ, GROVES RC, ET AL. Catabolic hormones and substrate patterns in septic patients. J Surg Res 23:177–182, 1977.
25. ARIEFF AI, and CARROLL HG. Non-ketotic hyperosmolar coma with hyperglycemia. Clinical fractures, pathophysiology, renal function, and base balance, plasma-cerebrospinal fluid equilibria and the effect of therapy in 37 cases. Medicine 51:73–94, 1972.
26. VAN OSS CJ, and BORDER JR. Influence of intermittent hyperglycemic glucose levels in the phagocytosis of microorganisms by human granulocytes in vitro. Immunol Commun 7:669–676, 1978.
27. CHANG S, and SILVIS SE. Fatty liver produced by hyperalimentation in rats. Am J Gastroenterol 62:410–418, 1974.
28. TEPPERMAN J, and TEPPERMAN HM. Effects of antecedent food intake pattern on hepatic lipogenesis. Am J Physiol 193:55–64, 1958.

Radionuclide Ejection Fraction in Doxorubicin Cardiotoxicity[1]

Graeme W. Morgan,* Brenda M. McIlveen, Albert Freedman, and I. Provan C. Murray[2,3]

SUMMARY

Serial assessment of cardiac function by radionuclide ejection fraction (EF), using a "first-pass" technique, was performed prior to each dose in 292 patients receiving doxorubicin. Detailed analysis was undertaken in 98 patients, consisting of all 91 patients who had received > 200 mg/m² and seven with risk factors (RFs) who had received between 100 and 200 mg/m². Thirty-two patients had RFs and 66 had no risk factors (NRFs). RFs were: (*a*) cardiovascular disease, (*b*) mediastinal irradiation within 6 months of starting doxorubicin treatment, and (*c*) total doxorubicin dose > 550 mg/m². The criteria for discontinuing doxorubicin were EF < 0.40 in RF patients or < 0.35 in NRF patients or decrease in EF of ≥ 0.14 over three successive readings. In the NRF group, no patient met these criteria nor did any develop cardiac failure. In the RF group, discontinuation was indicated in eight patients: two stopped receiving doxorubicin at total doses of 730 mg/m² and 680 mg/m², respectively, and it was discontinued in the other six at total doses ranging from 120 to 420 mg/m². Prior to the first dose of doxorubicin, all patients should have a baseline EF and assessment of RFs. An EF study is required before each subsequent dose in patients with RFs or with initial EF < 0.45, but in the remaining patients, serial studies are not required until there is a cumulative dose of 450 mg/m². Use of these guidelines and our criteria for discontinuation may prevent development of doxorubicin cardiotoxicity.

[Cancer Treat Rep 65:629–638, 1981]

Doxorubicin is an efficacious cytotoxic agent, but its use in malignant disease is limited by development of cardiomyopathy, with invariably fatal cardiac failure in a significant proportion of patients. Initial studies showed that the incidence of cardiotoxicity increased sharply above a cumulative dose of 550 mg/m², and it was therefore recommended that this dose should not be exceeded (1,2). However, there are reports of this side effect occurring at total doses well below this level (3,9) and of patients receiving > 1000 mg/m² without developing cardiac failure (4,13).

Attempts at predicting impending cardiotoxicity by ECG changes (2), systolic time intervals (5), echocardiography (6), endomyocardial biopsy (7) or prevention by prophylactic digoxin (8,10), coenzyme Q10 (9), steroids (10), vitamin E (11), or ICRF-159 (12) do not appear to offer advantage over limitation of total dose. We report our experience with serial evaluation of cardiac function by "first-pass" radionuclide ejection fraction to prevent development of irreversible cardiotoxicity in patients receiving doxorubicin therapy.

PATIENTS AND METHODS

Serial radionuclide ejection fraction (EF) studies

[1]Received June 3, 1980; revised Aug 22, 1980; accepted Sept 18, 1980.

[2]Institute of Oncology and Radiotherapy (*G. W. Morgan* and *A. Freedman*) and Department of Nuclear Medicine (*B. M. McIlveen* and *I. P. C. Murray*), The Prince of Wales Hospital, Randwick, Sydney, NSW Australia.

[3]We thank Prof. Atkinson and Drs. Coates, Grace, Himpoo, Heller, Lam-Po-Tang, Lavrin, and Rozenberg for allowing their patients to be studied; G. H. Molk for computer analysis; Dr. Fisher for statistical analysis; Dr. Hoschl for reviewing the manuscript; and Ms. B. Everist for secretarial assistance.

**Reprint requests to:* Dr. Graeme W. Morgan, MRCP, Institute of Oncology and Radiotherapy, The Prince of Wales Hospital, Randwick, Sydney, NSW Australia 2031.

were carried out in 292 patients. Detailed analysis was undertaken in 98 patients, consisting of all 91 patients who had received total doses of doxorubicin > 200 mg/m² and seven patients with risk factors who had received between 100 and 200 mg/m². In the remaining 194 patients the total dose was < 200 mg/m², and therefore, the number of serial EF studies was too small for meaningful analysis.

The study group consisted of 55 female and 43 male patients with an age range of 15–75 years (median, 52); 25 patients were > 60 years of age and three of these were > 70. The majority of histopathologic diagnoses were of non-Hodgkin's lymphoma, metastatic or recurrent breast carcinoma, bronchial carcinoma, or osteogenic or soft tissue sarcoma (table 1). Mediastinal irradiation had been given > 12 months previously to 17 patients with breast cancer. In ten patients who presented with bronchial carcinoma or superior vena cava (SVC) obstruction, doxorubicin and mediastinal irradiation were administered concurrently. Fifty-one patients received doxorubicin combinations including cyclophosphamide, and 47 patients received doxorubicin without cyclophosphamide.

Patients were evaluated by history, ECG, chest roentgenogram, and EF study plus cbc and biochemical screen prior to each doxorubicin injection (25–50 mg/m² every 3–4 weeks). EF analysis was performed without knowledge of doxorubicin dose or clinical state of the patient. In 14 patients, an EF study was only performed prior to every second dose, and serial evaluation was not started until the total dose was at least 250 mg/m² in ten additional patients, who subsequently had at least three serial studies.

Risk factors (RFs) were present in 32 patients, and 66 patients had no risk factors (NRFs). RFs were: (*a*) cardiovascular disease (angina, history of myocardial infarction, hypertension, or ECG evidence of left ventricular (LV) hypertrophy or old infarct); (*b*) mediastinal irradiation within 6 months of starting doxorubicin; and (*c*) a total dose of doxorubicin > 550 mg/m².

TABLE 1.—Histopathologic diagnosis in 98 patients evaluated by radionuclide EF*

Histopathologic diagnosis	NRF	RF	Total
Non-Hodgkin's lymphoma	26	7	33
Recurrent or metastatic breast carcinoma	15	2	17
Bronchial carcinoma	5	13	18
Soft tissue sarcoma	6	4	10
Osteogenic sarcoma	6	3	9
Other†	8	3	11
Total	66	32	98

*Values = No. of patients; NRF = no risk factor; RF = risk factor.

†Myeloma, acute myeloid leukemia, carcinoid syndrome, and stomach, kidney, and ovarian carcinoma.

The first-pass EF studies were performed using a gamma camera interfaced to a Digital Gamma-11 computer. After a rapid bolus iv injection of 600 MBq of ^{99m}Tc sodium pertechnetate, the study was stored on the computer in "list mode" for approximately 40 seconds. Recording was started when the bolus was seen on the gamma camera persistence scope to move into the right ventricle. The computer study was then framed in 40-millisecond frames, using a 64 × 64 matrix. LV-EF was calculated using the root mean square method of Schelbert et al (14). Interobserver (20 patients), intraobserver (ten patients), and temporal (ten stable patients) reproducibility studies were performed.

Our criteria for discontinuing doxorubicin were an EF < 0.40 with RFs present, an EF < 0.35 with NRFs, or a decrease in EF of ⩾ 0.14 over three successive readings (irrespective of total dose). Doxorubicin was also discontinued when there was disease progression, completion of treatment protocol, clinical evaluation of complete remission, or patient refusal to continue therapy.

RESULTS

Three types of change in EF were documented (fig 1): *type I* (62 patients)—no alteration in EF during serial evaluation (initial EF minus final EF ⩽ 0.07); *type II* (11 patients)—initial drop in EF (> 0.07 to ⩽ 0.14) in the first two or three readings, with no later change (subsequent EF minus final EF ⩽ 0.07); and *type III* (25 patients)—progressive, dose-related decline in EF (initial EF minus final EF > 0.07). The results are summarized according to cumulative doxorubicin dose and the presence or absence of RFs (table 2), the pattern of alteration in EF (table 3), and treatment using doxorubicin combinations with and without cyclophosphamide (table 4). EF prior to the first dose of doxorubicin was 0.57 ± 0.07 (mean ± SD). Reproducibility studies showed a mean interobserver absolute difference of 2% (20 studies), an intraobserver mean difference of 2.8% (ten studies), and an average coefficient of variation of 4.4% in serial studies in ten stable patients.

NRF Group

No patient without recent mediastinal irradiation or evidence of cardiovascular disease followed serially met our criteria for discontinuing doxorubicin, although in some there was slight fluctuation in EF

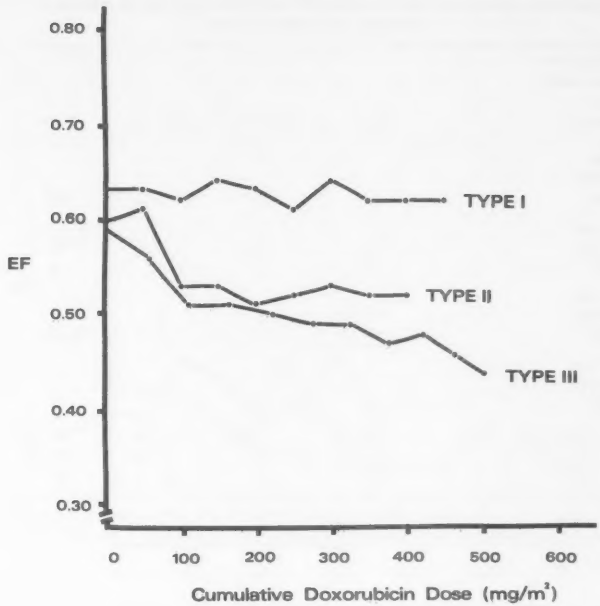

FIGURE 1.—Patterns of change in EF with serial study: type I, 64-year-old male with non-Hodgkin's lymphoma; type II, 42-year-old female with soft tissue sarcoma; and type III, 58-year-old female with metastatic breast carcinoma.

(fig 2). Most of the patients (55 of 66, 83.3%) showed little variation in EF (type I or II). The percent of patients who developed marked change in EF (type III) increased from 2.6% (one of 38 patients who received total doses < 350 mg/m²) to 35.7% (ten of 28 patients who received doses > 350 mg/m²). Likewise, in patients who had received a direct anterior field to the internal mammary chain (IMC) > 12 months previously during postoperative irradiation for breast carcinoma, the percent of patients with type III change increased from none of ten patients who received total doses < 350 mg/m² to four of five patients who received doses > 350 mg/m². The distribution of patients according to change in EF was similar for patients receiving doxorubicin combinations with and without cyclophosphamide (table 4).

Eight patients had an initial EF < 0.45 but re-

TABLE 2.—Distribution of NRF and RF patients according to cumulative doxorubicin dose*

Cumulative doxorubicin dose (mg/m²)	NRF	RF	Total
> 550		11 (1)	11
350–549	28 (5)	5	33
200–349	38 (10)	9 (1)	47
100–199	NE	7	7
Total	66 (15)	32 (2)	98

*Values = No. of patients; values in parentheses = No. of patients with breast cancer who had received postoperative mediastinal irradiation > 12 mos previously; NE = not evaluated.

TABLE 3.—Pattern of EF change in RF and NRF patients in relation to cumulative doxorubicin dose*

Cumulative doxorubicin dose (mg/m^2)	Type I	Type II	Type III	Total
> 550				
NRF				
RF	4	2	5 (1)	11
350–549				
NRF	14	4 (1)	10 (4)	28
RF	3	1	1	5
200–349				
NRF	34 (10)	3	1	38
RF	5 (1)	1	3	9
100–199				
NRF				
RF	2		5	7
Total NRF	48 (10)	7 (1)	11 (4)	66 (15)
Total RF	14 (1)	4	14 (1)	32 (2)
Total	62	11	25	98

*Values = No. of patients; values in parentheses = No. of patients with breast cancer who had received postoperative mediastinal irradiation > 12 mos previously.

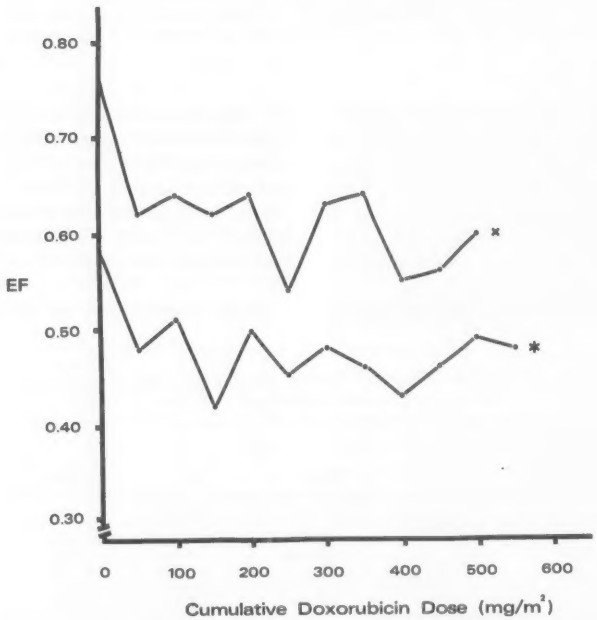

FIGURE 2.—Variations in EF with serial study: x = 52-year-old female with metastatic breast carcinoma; * = 56-year-old male with non-Hodgkin's lymphoma.

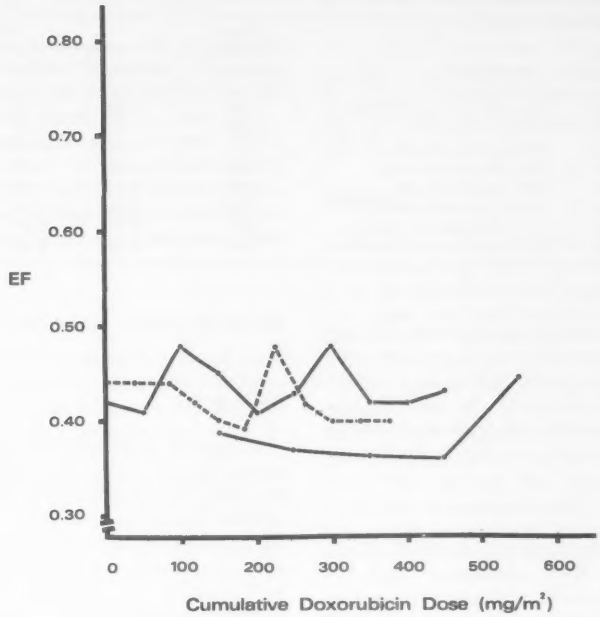

FIGURE 3.—Serial EF studies in 3 NRF patients with initial EF < 0.45. (*See* table 5).

TABLE 4.—Pattern of EF change in patients receiving doxorubicin combinations with and without cyclophosphamide*

Cumulative doxorubicin dose (mg/m²)	Type I	Type II	Type III	Total
> 550				
D	3	2	3	8
D + C	1		2	3
350–549				
D	6	3	5	14
D + C	11	2	6	19
200–349				
D	23	1	1	25
D + C	16	3	3	22
100–199				
D				
D + C	2		5	7
Total D	32	6	9	47
Total D + C	30	5	16	51
Total	62	11	25	98

*Values = No. of patients treated with doxorubicin combinations with (D + C) and without (D) cyclophosphamide.

TABLE 5.—EF values in patients with initial EF < 0.45 (NRF)

Patient	Sex	Age (yrs)	Histopathologic diagnosis	Final cumulative dose (mg/m²)	EF values			
					Initial	Final	Maximum	Minimum
A	F	21	Non-Hodgkin's lymphoma	540	0.39	0.45	0.45	0.36
B	M	56	Lung carcinoma	250	0.42	0.53	0.56	0.42
C	M	21	Osteogenic sarcoma	275	0.44	0.43	0.47	0.43
E	F	32	Soft tisssue sarcoma	365	0.44	0.40	0.44	0.39
F	F	25	Soft tissue sarcoma	445	0.42	0.43	0.48	0.40
G	M	40	Acute myeloid leukemia	300	0.43	0.44	0.44	0.42
H	F	65	Non-Hodgkin's lymphoma	225	0.42	0.39	0.50	0.39
I	F	62	Recurrent breast carcinoma	205	0.44	0.43	0.44	0.41

ceived total doses of doxorubicin between 205 and 540 mg/m² without development of cardiotoxicity (table 5). Three of these patients had EFs < 0.40, but as no RFs were present and the need for continued treatment outweighed the risk of cardiotoxicity, additional doxorubicin was given (fig 3).

RF Group

RFs were identified in 32 patients (table 6). Three patients had multiple RFs: cumulative dose and hypertension, atherosclerosis and recent irradiation (patient 4; *see* Case Reports), and hypertension and irradiation (patient 2). As with NRF patients, patterns of change in EF were unpredictable. However, type III change was documented in 43.75% (14 of 32) of the RF patients and in only 16.7% (11 of 66) of the NRF patients. This difference in type III change has high statistical significance ($P = 0.006$). In patients receiving < 550 mg/m², the type III change is also highly significant: nine of 21 patients with RF and 11 of 66 patients with NRF ($P = 0.019$, χ^2 test). Eight of the 32 patients (25%) had doxorubicin discontinued as our criteria were met. Cardiac failure occurred in three patients, with subsequent death in one of these, but clinical evidence of cardiotoxicity did not develop in the other five.

CASE REPORTS

Patient 1

Patient 1, a 58-year-old male with carcinoid syndrome, had an EF of 0.68 in the first EF study, when he had received 580 mg/m² of doxorubicin. In the third study (total dose, 680 mg/m²), doxorubicin was discontinued as EF had fallen to 0.52. Two months later, cardiac failure developed at an EF of 0.48. Heart size was normal as observed on chest roentgenogram, and there was no voltage reduction on ECG. Sudden death occurred 6 weeks later. The RF in this patient was a total dose > 550 mg/m² (fig 4).

Patient 2

Patient 2, a 42-year-old male with primary adenocarcinoma of the lung, developed cardiac failure at a total dose of 240 mg/m² (EF, 0.25). No previous studies had been performed. He was treated with two cycles of cyclophosphamide, doxorubicin, methotrexate, and procarbazine and a split course of irradiation to the primary and the mediastinum (parallel opposed fields, using 6-MeV photons to deliver an 18.8-Gy midplane dose (MPD) in eight fractions over 12 days on each occasion), with cycle 3 intervening and cycle 4 given after completion of irradiation. The EF study was performed only because cardiac failure developed. There was clinical evidence of cardiomyopathy 5 months after doxorubicin was discontinued. RFs were hypertension, LV hypertrophy, cardiomegaly on chest roentgenogram, and concurrent mediastinal irradiation. These RFs were noted in retrospect. It is possible that if they had been recognized and if EF studies had been performed, cardiac failure may have been avoided.

TABLE 6.—Distribution of RF in patients in RF group

Risk factor	No. of patients
Cardiovascular disease	
Angina on effort	3
History of infarct	2
Hypertension	2
ECG	
LV hypertrophy	2
Old infarct	2
Mediastinal irradiation*	10
Total dose > 550 mg/m²	11
Total	32

*Within 6 mos of starting doxorubicin therapy.

Patient 3

Patient 3, a 35-year-old female with soft tissue sarcoma, had an EF of 0.72 in the second EF study, at a total dose of 650 mg/m² of doxorubicin; the EF decreased to 0.51 in the fourth study (total dose, 730 mg/m²), and doxorubicin was stopped. Four months later, the EF was 0.52. The patient died of progressive disease without clinical evidence of cardiac failure 8 months after doxorubicin was discontinued. The RF in this patient was a total dose > 550 mg/m² (fig 4).

Patient 4

Patient 4, a 64-year-old male with oat-cell lung carcinoma, presented with SVC obstruction in September 1977 and was treated with irradiation (parallel opposed fields to the mediastinum, using 6-MeV photons to deliver 28 Gy MPD in 12 fractions over 18 days). The diagnosis was confirmed by supraclavicular node biopsy in November 1977. Cytotoxic treatment with vincristine, cyclophosphamide, and doxorubicin (OCA) was started. Doxorubicin was discontinued after a total dose of 120 mg/m² (EF, 0.39). EF improved to 0.52, with monthly evaluations over 5 months. The patient died of progressive disease without clinical evidence of cardiac failure. The RFs were ECG evidence of old myocardial infarction and mediastinal irradiation 3 months prior to the start of doxorubicin treatment (fig 4).

Patient 5

Patient 5, a 52-year-old male with oat-cell lung carcinoma, was treated with three cycles of cytotoxic agents (OCA) followed by a split course of irradiation to the primary and mediastinum (parallel opposed fields, using 6-MeV photons to deliver an 18.8-Gy MPD in eight fractions over 12 days on each occasion), with cycle 4 of OCA intervening. The EF was 0.46 prior to the first dose of doxorubicin, and following irradiation it was 0.39 (total dose, 130 mg/m²). Doxorubicin was withheld, and a followup EF was not obtained. There was no clinical evidence of cardiac failure 8 months after doxorubicin was discontinued. The RF was concurrent mediastinal irradiation (fig 4).

Patient 6

Patient 6, a 58-year-old female with oat-cell lung carcinoma, was treated with three weekly cycles of cytotoxic agents (OCA), without mediastinal irradiation. The EF was 0.60 prior to the second dose of doxorubicin; prior to fourth and fifth doses, it was 0.45; doxorubicin was discontinued prior to the sixth dose (total dose, 240 mg/m²; EF, 0.36). One month later, EF was 0.47. There was no clinical evidence of cardiac failure 4 months after doxorubicin was discontinued. The RF was angina on effort (fig 4).

Patient 7

Patient 7, a 42-year-old male with oat-cell lung carcinoma, was treated with three cycles of cytotoxic agents (OCA) and a split course of irradiation to primary and mediastinum (parallel opposed fields, using 6-MeV photons to deliver an 18.8-Gy MPD in eight fractions over 12 days on each occasion), with cycle 4 intervening. Initial EF was 0.46 prior to the second dose, and it was 0.41 prior to the third and fourth doses. Doxorubicin was discontinued prior to the fifth dose, when the EF decreased to 0.37 (total dose, 160 mg/m²). One month later, the EF was 0.42. There was no clinical evidence of cardiac failure at followup 6 months later. The RF was concurrent mediastinal irradiation (fig 4).

Patient 8

Patient 8, a 56-year-old female with metastatic breast carcinoma, was treated with three weekly cycles of doxorubicin (30 mg/m²) and cyclophosphamide. EF studies were not performed prior to each dose, but pretreatment EF was 0.53, and at a total dose of 300 mg/m², EF was 0.46. The next EF was 0.33 at a total dose of 420 mg/m². An additional dose of 30 mg/m² was given, and 3 weeks later, the patient presented in cardiac failure (EF, 0.32). The congestive cardiac failure was controlled by standard methods, although EF remained depressed at 0.28. RFs were hypertension for 5 years, controlled with diuretic and beta-blocker therapy; ECG evidence of LV hypertrophy; and fundi-grade II retinopathy. Cardiotoxicity may have been prevented if RFs had been noted and serial studies had been performed. Inadvertently, an additional dose of doxorubicin was given when the EF was 0.33.

DISCUSSION

The search for a method of predicting doxorubicin-induced cardiotoxicity has previously been unrewarding. The standard clinical practice of limiting total dose to 550 mg/m² (or 450 mg/m² with prior mediastinal irradiation or concomitant cyclophosphamide therapy) (1,2) ignores reports of cardiac failure occurring in some patients at doses far below these levels (3,9) and lack of toxic effects at doses

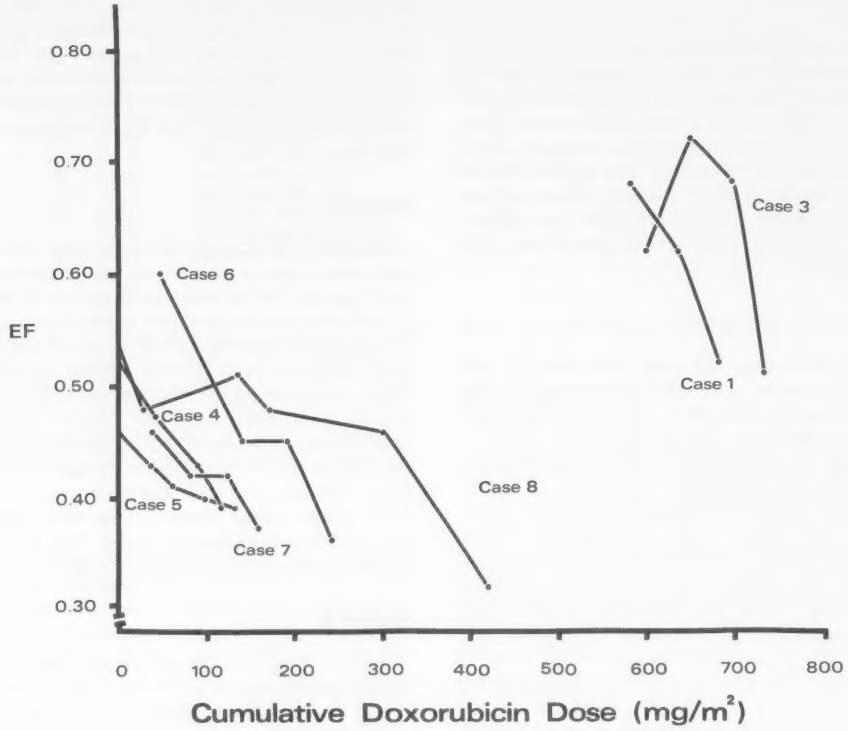

FIGURE 4.—Serial EF studies in patients who satisfied criteria for discontinuation of doxorubicin.

> 1000 mg/m² (4,13) in others. Limitation of total dose does not take into account physiologic variation or reports that cardiotoxicity may be related to dose scheduling (4,13), and the drug may therefore be denied to some patients who could receive additional therapy with benefit. A suitable predictive method would allow for the drug to be safely administered in excess of 550 mg/m² when clinically indicated but would prevent administration of additional doxorubicin at low total doses when cardiac decompensation was likely to lead to iatrogenic death.

The radionuclide estimation of EF is a simple, reproducible, noninvasive method of assessing cardiac function (14,15,20). Use of this method in predicting doxorubicin-induced cardiotoxicity has been previously reported (16–19). There are two methods for assessing ejection fraction using radionuclides: "first pass" and "gated equilibrium" (20). The former provides a more rapid measurement of EF, but, unlike the latter, it does not readily permit study of regional wall motion. Both are well recognized for assessing ventricular function in coronary artery disease and as a prognostic index prior to coronary artery bypass surgery (21). EF after exercise stress testing (22) and the first third EF (23) are modifications which increase the sensitivity of this method in detecting early myocardial damage in ischemic heart disease and may therefore have application in doxorubicin-induced cardiac decompensation. However, difficulties may be encountered in achieving maximum or adequate stress, considering the clinical state of many patients with malignant disease, in addition to the extra time needed. The first-pass technique was chosen for this study because of the speed and accuracy with which results could be obtained for a large number of patients and because study of wall motion did not seem likely to provide additional information relevant to the clinical problem.

Criteria for discontinuing doxorubicin were based on reports of poor survival rates in patients undergoing coronary artery bypass surgery with a preoperative angiographic EF < 0.30 (21). Initially, our cutoff point was 0.45, but with experience in

the eight patients with initial EF below 0.45, the lower limit was reduced to 0.40 in patients with RFs and to 0.35 in those with NRFs. These values have been confirmed as appropriate by Alexander et al (19), who found that doxorubicin-induced cardiac failure invariably occurred with an EF < 0.30, and they are supported by the case report on patient 2 in this study.

In addition, rapid decrease in EF over three successive readings was considered to represent failure of cardiac muscle to withstand previous doxorubicin insults. Two standard deviations from the mean (0.14) was arbitrarily considered to reflect cardiac decompensation. This criterion was applied to patients 1 and 3, but as seen in patient 1, cardiac failure occurred nevertheless at an EF of 0.48. This is in conflict with the proposal of Alexander et al (19), who would not stop administration of doxorubicin after a decrease of 0.15 unless the EF was also < 0.45. Since serial EF studies may show some fluctuation (fig 2), three successive studies were needed to document sustained decrease in EF. Further, we would repeat an EF study in patients with values between 0.35 and 0.40 before discontinuing doxorubicin. As improvement in EF can occur after doxorubicin is discontinued, the repeat study should be carried out within 1 week.

In evaluating RFs, mediastinal irradiation was studied closely. As originally suggested by Merrill et al (24), irradiation and doxorubicin applied with short-term temporal separation are synergistic. In our series, two groups of patients received mediastinal irradiation. Ten patients presenting with bronchial carcinoma or SVC obstruction received mediastinal irradiation concurrently or within 6 months of starting doxorubicin, and in four of these patients, the criteria for discontinuation were met. In these patients, the primary lesion and mediastinum were irradiated through anterior and posterior parallel opposed fields to deliver a total MPD either during a single treatment regimen or as a split course with chemotherapy intervening. No patient received > 40 Gy MPD in 4–6 weeks (cardiac dose). The second group of 17 patients had received a direct IMC field during postoperative irradiation for breast carcinoma > 12 months previously. On serial study, only patient No. 8 met the criteria for discontinuation of doxorubicin. A 4-MeV linear accelerator was used to deliver 45 Gy at a depth of 3 cm, in 25 fractions over 5 weeks, resulting in 32 Gy MPD (cardiac dose). If tangential fields were used to treat the chest wall, this would not increase the MPD.

Our results confirm that irradiation and doxorubicin given in close sequence constitute a risk factor but that mediastinal irradiation > 12 months previously does not necessarily require a reduction in cumulative doxorubicin dose. The practice of treating breast cancer postoperatively with tangential fields to include both the IMC and chest wall has been shown by Fletcher and Montague (25) to give a variable mediastinal dose but far less than that delivered by a direct IMC field. The failure to appreciate this difference in technique may be the reason for conflicting reports of the need to limit the total dose of doxorubicin to 450 mg/m² with prior mediastinal irradiation (2,13).

Pre-existing cardiovascular disease is an obvious RF. With an already compromised cardiac state, a cardiotoxic agent may give rise to complications more readily. Four of 11 patients with evidence of cardiovascular disease, two of whom received concurrent mediastinal irradiation, required discontinuation of doxorubicin compared with two of 74 patients without either factor present.

Cumulative dose > 550 mg/m² is also an RF, as predicted from previous clinical studies; this is confirmed in the case reports on patients 1 and 3. However, six of 11 patients in the RF group with doses ranging from 550 to 800 mg/m² showed little variation in EF (types I and II). This is consistent with the data of Von Hoff et al (13) that there is a continuum of increasing risk of cardiotoxicity as the cumulative dose of doxorubicin increases. Likewise, we have been unable to confirm that concomitant administration of cyclophosphamide is an RF necessitating that the cumulative dose be reduced to 450 mg/m².

In 73 of the 98 patients in the study group (74.5%) the serial EF studies showed no changes related to the dose of doxorubicin (type I or II). Therefore, serial EF studies are needed only in certain circumstances. Although 11 of 66 patients in the NRF group showed type III change in EF, the criteria for discontinuing doxorubicin were not met. We therefore feel that in patients without RFs and with an initial EF > 0.45, serial studies are not required until a cumulative dose of 450 mg/m² is received. Thus, when the total dose reaches 550 mg/m², at least three studies will have been performed and, provided our criteria for cessation are not met, additional doxorubicin could be given if indicated.

In the RF group, eight of 32 patients (25%) met our criteria and had doxorubicin discontinued. In six patients, this occurred at low total doses: 240, 120, 130, 240, 160, and 450 mg/m² (patients No. 2, 4, 5, 6, 7, and 8, respectively). Particularly as three of the patients had normal initial EF values, we consider that our experience with these patients justifies pretreatment and serial EF studies prior to every doxorubicin dose, in all patients with risk factors. In particular, cardiac failure might have

been avoided in patients 2 and 8 if this had been carried out. We therefore differ from Alexander et al (19), who suggest that, provided a baseline EF obtained before a cumulative dose of 350 mg/m^2 is normal, a second EF is not needed until a total dose of 400 mg/m^2 has been given, even in patients with known heart disease. In addition, serial EF studies are advisable when the initial value is < 0.45, even if no RFs are present, despite our experience to date showing that doxorubicin can be safely given with careful monitoring. Such patients have an initial EF value which is abnormal and indicative of LV dysfunction and should therefore be followed closely.

Our data support the concept that an RF group warrants careful monitoring and that an NRF group does not require EF studies until a threshold cumulative dose is reached. As patients with an abnormal EF (< 0.50) may continue to receive doxorubicin without risk of cardiac failure, we have attempted to improve the usefulness of the EF method by adopting criteria for discontinuation. Because of ethical considerations, we have been unable to show unequivocally that patients with depressed EF or rapid decrease in EF would develop cardiac failure with continued doxorubicin treatment. Thus, our definition of RF patients and criteria for discontinuation may be modified by subsequent experience and publications. However, like Alexander, we believe radionuclide EF is of immense value to physicians treating patients with doxorubicin and that use of these guidelines and our criteria for discontinuation may prevent development of cardiotoxicity.

REFERENCES

1. Lefrak EA, Pitha J, Rosenheim S, et al. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 32:302–314, 1973.
2. Minow RA, Benjamin RS, and Gottlieb JA. Adriamycin (NSC-123127) cardiomyopathy—an overview with determination of risk factors. Cancer Chemother Rep Part 3, vol 6(2):195–201, 1975.
3. O'Bryan RM, Baker LH, Gottlieb JA, et al. Dose response evaluation of adriamycin in human neoplasia. Cancer 39:1940–1948, 1977.
4. Weiss AJ, and Manthel RW. Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule, with particular reference to lack of cardiac toxicity. Cancer 40:2046–2052, 1977.
5. Rinehart JJ, Lewis RP, and Balcerzak SP. Adriamycin cardiotoxicity in man. Ann Intern Med 81:475–487, 1974.
6. Bloom KR, Bini RM, Williams CN, et al. Echocardiography in adriamycin cardiotoxicity. Cancer 41:1265–1269, 1978.
7. Bristow MR, Mason JW, Billingham ME, et al. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy and cardiac catheterization. Ann Intern Med 88:168–175, 1978.
8. Williams CJ. Doxorubicin cardiotoxicity: role of digoxin in prevention. Br Med J 1:176, 1978.
9. Cortes EP, Gupta M, Chou C, et al. Adriamycin cardiotoxicity: early detection by systolic time interval and possible prevention by coenzyme Q10. Cancer Treat Rep 62:887–891, 1978.
10. Gupta M, Cortes EP, and Mundia A. Systolic time interval (STI) in adriamycin (ADM) treated patients on digoxin or prednisone cardioprophylaxis. Proc Am Assoc Cancer Res and ASCO 17:269, 1976.
11. McGuire W, Young RC, Grotzinger K, et al. Effects of tocopherol on adriamycin-induced cardiomyopathy and tumour response. Proc Am Assoc Cancer Res and ASCO 18:85, 1977.
12. Wang GM, and Finch M. Studies on the mechanisms of razoxane induced reduction of daunomycin toxicity. Proc Am Assoc Cancer Res and ASCO 20:23, 1979.
13. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91:710–717, 1979.
14. Schelbert HR, Verba JW, Johnson AD, et al. Nontraumatic determination of left ventricular ejection fraction by radionuclide angiocardiography. Circulation 51:902–909, 1975.
15. Trobaugh GB, Kennedy JW, Hamilton GW, et al. Reproducibility of ventricular function measurements by angiographic and radionuclide angiography. Am J Cardiol 41:439, 1978.
16. Morgan GW, Choy D, and Freedman A. Radionuclide ejection fraction and prediction of doxorubicin cardiotoxicity. Lancet I:1315–1316, 1978.
17. Singer JW, Narahara KA, Ritchie JL, et al. Time- and dose-dependent changes in ejection fraction determined by radionuclide angiography after anthracycline therapy. Cancer Treat Rep 62:945–948, 1978.
18. Morgan G, Freedman A, Molk GH, et al. Radionuclide ejection fraction and prediction of adriamycin cardiotoxicity. Proc Am Assoc Cancer Res and ASCO 20:425, 1979.
19. Alexander J, Dainiak N, Berger HJ, et al. Serial assessment of doxorubicin cardiotoxicity with quantitative radionuclide angiocardiography. N Engl J Med 300:278–283, 1979.
20. Ashburn WL, Schelbert HR, and Verba JW. Left ventricular ejection fraction—a review of several radionuclide angiographic approaches using the scintillation camera. Prog Cardiovasc Dis 20:267–284, 1978.
21. Cohn PR, Forlin R, Cohn LH, et al. Left ventricular ejection fraction as a prognostic guide in surgical treatment of coronary and valvular heart disease. Am J Cardiol 34:136–141, 1974.
22. Brady TJ, Thrall JH, Clare JM, et al. Exercise radionuclide ventriculography: practical considerations and sensitivity in coronary artery disease detection. Radiology 132:697–702, 1979.
23. Slutsky R, Gordon D, Karliner J, et al. Assessment of early ventricular systole by first pass radionuclide angiography: useful method for detection of left ventricular dysfunction at rest in patients with coronary artery disease. Am J Cardiol 44:459–465, 1979.
24. Merrill J, Greco FA, Zimbler H, et al. Adriamycin and radiation: synergistic cardiotoxicity. Ann Intern Med 82:122–123, 1975.
25. Fletcher GH, and Montague ED. Does adequate irradiation of the internal mammary chain and supraclavicular nodes improve survival rates. Int J Radiat Oncol Biol Phys 4:481–492, 1978.

Intensive Induction Chemotherapy in 54 Patients With Small Cell Carcinoma of the Lung [1,2]

Martin D. Abeloff,* David S. Ettinger, Stanley E. Order, Nagi Khouri, E. David Mellits, Neal T. Dorschel, and Rosemary Baumgardner [3,4]

SUMMARY

Fifty-four patients with localized and extensive small cell carcinoma of the lung and no prior therapy were treated with intensive induction chemotherapy. The induction regimen consisted of two courses of high-dose cyclophosphamide, doxorubicin, and VP-16-213. The objective response rate was 78% (42 responses among 54 patients), with 14 complete (26%) and 28 partial (52%) responses. The median survival time for the entire group of patients is 378 days, with a projected 2-year survival rate of 22%. The most significant determinant of survival was the attainment of a complete response. Tumor progression in most relapsing patients occurred at the sites of initial involvement. Toxicity was significant, as expected, but treatment-related mortality did not exceed that of less intense regimens. Despite the use of such intensive induction regimens, the major challenge in the therapy for small cell carcinoma remains the achievement of more durable complete remissions.

[Cancer Treat Rep 65:639–646, 1981]

During the past 10 years, the utilization of combination chemotherapy with or without radiotherapy has led to significant increases in survival for patients with small cell carcinoma of the lung (SCC) (1,2). The therapeutic gains achieved with standard-dose systemic chemotherapy have subsequently served as the basis for the study of more intensive chemotherapeutic approaches.

The early therapeutic results from these intensive experimental studies were encouraging (3), but the superiority of such aggressive treatment regimens over standard-dose chemotherapy has not been firmly established. Based on data regarding the safety and efficacy of high-dose chemotherapy in SCC (4), we designed studies in 1977 to evaluate the effectiveness and toxicity of very intensive induction chemotherapy with cyclophosphamide, doxorubicin, and VP-16-213 (CAV) in patients with localized and extensive SCC. We have previously reported our preliminary results with these regimens (5,6); this paper describes in detail the response data and toxicity in 54 patients treated with this intensive chemotherapeutic approach and critically assesses the risk-benefit ratio of such therapy.

MATERIALS AND METHODS

Patient Selection and Therapy

Fifty-four previously untreated patients with SCC were entered in the study at The Johns Hopkins Oncology Center between September 1977 and June 1979. Following diagnostic workup, 20 patients were classified as having localized disease and 34 patients

[1] Received July 14, 1980; revised Nov 18, 1980; accepted Nov 26, 1980.

[2] Supported in part by Public Health Service grant CA-06973 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] The Johns Hopkins Comprehensive Cancer Center, Departments of Radiology and Pediatrics, The Johns Hopkins Hospital, Baltimore, MD.

[4] We gratefully acknowledge the collaboration of The Johns Hopkins Cancer Center Clinical Information Systems in the collection, calculation, and tabulation of the data presented in this paper. The excellent secretarial assistance of Nancy B. Kramer and Mimi Yacoub is also acknowledged.

**Reprint requests to:* Martin D. Abeloff, MD, The Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—Stage of disease, PS, and response to therapy

Stage	Total No. of patients	No. of patients with CR	No. of patients with PR or no response	
Extensive				
Fully ambulatory*	14	5	9	
Less than fully ambulatory†	20	1	19	
Localized				
Fully ambulatory	10	6	4	
Less than fully ambulatory	10	2	8	
Localized and extensive				
Fully ambulatory	24	11	13	‡
Less than fully ambulatory	30	3	27	‡

*ECOG PS 0 or 1.
†ECOG PS 2, 3, or 4.
‡$P < 0.01$.

were staged as having extensive disease. Localized disease was defined as disease confined to one hemithorax including ipsilateral hilar and mediastinal nodes. Extensive disease was defined as disease beyond the hemithorax; patients with ipsilateral supraclavicular nodes, heterolateral mediastinal or hilar nodes, superior vena cava (SVC) syndrome, and pleural effusion (regardless of cytologic findings) were classified as having an extensive stage. Patients with central nervous system metastases at the time of diagnosis were not included in this study.

The median age at diagnosis of the 54 patients in this study was 59 years (range, 41–69). There were 35 males and 19 females. Forty-six of the patients were white and eight were black. All patients were included in analysis of response and survival. The Eastern Cooperative Oncology Group (ECOG) performance status (PS) of this patient population at the initiation of therapy is described in table 1. Only one (6%) of the patients with localized disease was confined to bed for > 50% of the time (PS 3), whereas 15 (44%) patients with extensive disease had a PS of 3 or were totally bedridden (PS 4).

The sites of metastases at the time of diagnosis in patients with extensive disease are described in table 2. Seven of the 34 patients (21%) presented with SVC syndrome. Five patients were classified as having extensive disease on the basis of heterolateral mediastinal or hilar nodes or SVC syndrome. Only two patients had ipsilateral supraclavicular adenopathy as the only site of metastatic disease, while one patient had pleural involvement and one had marrow invasion as the sole sites of extensive disease.

The therapeutic protocol for patients with extensive SCC consisted of two courses of CAV (cyclophosphamide, 2400 mg/m² iv; doxorubicin, 60 mg/m² iv on Day 1; and VP-16-213, 125 mg/m² iv on Days 1, 3, and 5) administered at 28-day intervals while the patients were in the hospital. On Day 56, the patients started receiving BOMP on an outpatient basis (BCNU, 60 mg/m² iv; vincristine, 1.4 mg/m² [maximum, 2.0 mg] iv; methotrexate, 30 mg/m² iv on Day 56; and procarbazine, 100 mg/m²/day orally from Day 56 to Day 65). The vincristine and methotrexate were repeated on Day 70, and a second course of BOMP was started on Day 84. On Day 112 of the study, the patient's response status was reassessed. Patients with complete response (CR) or partial response (PR) were scheduled for prophylactic cranial radiotherapy (2100 rads in seven fractions) and then received an outpatient modifi-

TABLE 2.—Sites of metastases at time of diagnosis in 34 patients with extensive SCC

Site	No. of patients	% of patients with extensive disease
Marrow	16	47
Liver	15	44
Pleura	14	41
Bone	13	38
Ipsilateral supraclavicular node	11	32
Heterolateral chest node	11	32
Cervical and/or axillary node	5	15
Heterolateral lung	1	3
Subcutaneous nodule	1	3
Choroid	1	3
Pancreas	1	3
Thyroid	1	3

cation of CAV (cyclophosphamide, 1000 mg/m² iv; doxorubicin, 40 mg/m² iv on Day 1, and VP-16-213, 100 mg/m² iv on Days 1, 3, and 5) alternating at monthly intervals with BOMP. Treatment of complete responders was stopped at 2 years, and partial responders continued receiving therapy until disease progression occurred. Nonresponders and patients with disease progression received individualized therapy.

Following the entry of 17 extensive-stage patients in the study, it became apparent that the BOMP regimen did not significantly contribute to the response rate, so outpatient CAV was substituted for BOMP in the next 15 patients with extensive disease. Two patients with extensive disease were evaluable only for their response to the two intensive induction CAV courses; one died 2 days following his second course of intensive CAV, and the other had disease progression within 2 weeks of the second course of intensive CAV and was taken off protocol.

Patients with localized disease were randomized to receive chemotherapy only or chemotherapy plus radiotherapy to the primary tumor. The chemotherapy regimens were identical to the treatment given to patients with extensive disease, with the exception that the first course of CAV was given at outpatient doses in the combined radiotherapy-chemotherapy treatment. Radiotherapy to the primary tumor was administered in a split course. Two thousand rads in five fractions were administered on Days 1–5. Following the first course of CAV, 1500 rads in five fractions were delivered on Days 22–26. The second part of the split course of radiotherapy was frequently delayed 1 week because of leukopenia. Utilization of a treatment simulator and fluoroscopy enabled the delineation of a treatment portal that avoided the esophagus while delivering high-dose radiation to the primary tumor. Complete and partial responders on both arms of the localized-disease study received prophylactic cranial radiotherapy.

Assessment and Analysis of Response

Assessment of response was made 4 weeks after each course of chemotherapy. All measurements of tumor size on chest roentgenogram were made by one chest radiologist (N. Khouri). Approximately 4 months after initiation of chemotherapy, all sites of initial tumor involvement were assessed with appropriate laboratory and radiologic tests. Patients with CRs were not routinely re-examined with bronchoscopy.

Standard ECOG response criteria were utilized. Duration of response and survival were calculated from the first day of chemotherapy. Statistical comparison of response rate, PS, extent of disease, and specific sites of metastases involved the standard chi-square test with Yates' correction. Duration of response and survival plots were analyzed by the actuarial method of Kaplan and Meier (7). Comparisons of these curves utilized the tests of Peto et al (8).

RESULTS

Response Rate and Duration

Of 54 patients, 42 (78%) achieved an objective response to chemotherapy but a CR was seen in only 14 (26%). Eight of 20 (40%) patients with localized disease and six of 34 (18%) with extensive disease achieved a CR. The maximal antitumor response generally occurred during intensive induction therapy. As shown in table 1, the PS is related to type of response ($P < 0.01$ by chi-square analysis). There was no significant difference in the response rate achieved in patients with extensive disease who received BOMP chemotherapy as compared to those who continued to receive chemotherapy with CAV. Six of 11 (55%) patients with localized disease who received chemotherapy alone achieved a CR while only two of nine (22%) patients who received chest radiotherapy plus chemotherapy were classified as achieving a CR. This difference in CR may largely reflect difficulties in interpreting chest radiographs following radiotherapy since the survival in the two arms of the localized disease study was equivalent.

The duration of response for the complete responders is significantly longer than that of the partial responders at $P < 0.006$. The median duration of CR and PR is 421 and 224 days, respectively. Five of the patients with localized disease are currently in remission from 251 to 849 days following initiation of therapy while six patients with extensive disease remain in remission from 328 to 988 days.

Survival

Utilizing Kaplan-Meier survival analysis, the estimated median survival time for the entire group of 54 patients is 378 days, with a projected 2-year survival rate of 22%. Complete responders have a significantly longer survival time than partial responders and nonresponders at $P < 0.002$. The median survival time for complete responders was 752 days, and for partial responders and nonresponders it was 289 days. Although the median survival time

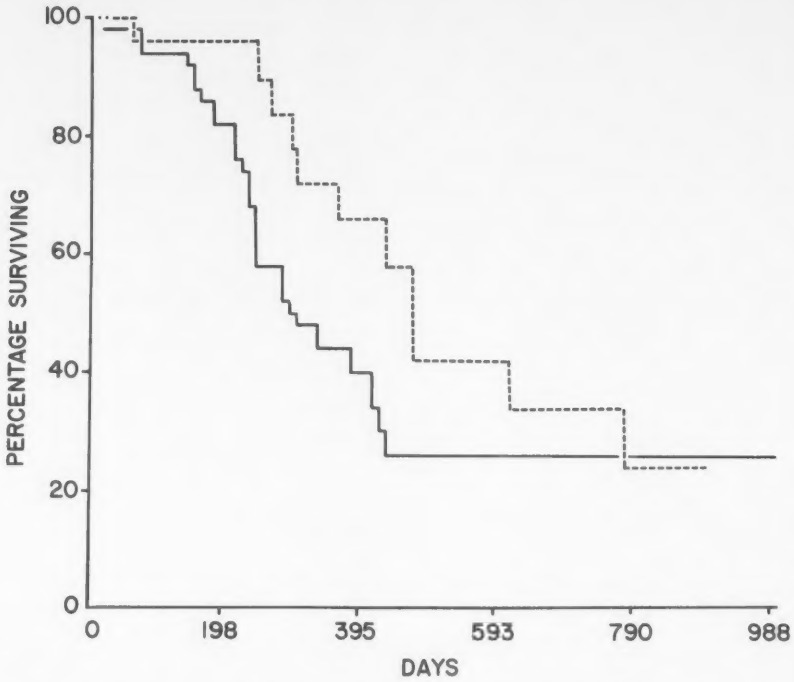

FIGURE 1.—Survival duration for patients with localized (dashed line) and extensive (solid line) disease.

for patients with localized and extensive disease is 465 and 290 days, respectively, the survival curve for patients with localized disease is not significantly different from that of patients with extensive disease (fig 1). Furthermore, patients with localized disease who achieve CR do not have an overall survival curve significantly different than patients with extensive disease who achieve CR (fig 2).

PS, use of chest radiotherapy in patients with localized disease, and use of BOMP chemotherapy in patients with extensive disease did not affect survival to a statistically significant degree. In addition, we were unable to identify "favorable" or "unfavorable" sites of metastases prior to treatment, and the number of metastatic sites did not significantly influence survival within the group of patients with extensive disease. However, the effect of these factors on survival may not be demonstrable in a patient population of this size.

Sites of Relapse and Residual Disease

Evaluation of the patients with localized disease for initial relapse sites reveals that six of the 11 relapses (55%) occurred solely at the site of the primary tumor. Only one patient who received chest radiotherapy relapsed at the site of the primary tumor. However, nine of the patients who relapsed have died and none died as a result of progression of the primary tumor.

One patient with localized disease who achieved a CR while receiving chemotherapy alone developed a second primary lung cancer (squamous cell carcinoma) and died 606 days following the first therapy for SCC. Two patients with localized disease did not respond to therapy. One of these patients remains alive with persistent chest tumor (confirmed by biopsy) and continued left upper lobe atelectasis but with no distant metastases 888 days after first therapy. The second nonresponder had 40% reduction in the chest tumor and no evidence of distant spread; he underwent a thoracotomy that revealed no evidence of SCC but did show inoperable adenocarcinoma. He died of brain metastases 427 days following first therapy for SCC. One patient died during induction therapy as a result of massive pulmonary infarction and infection.

Evaluation of the patients with extensive disease

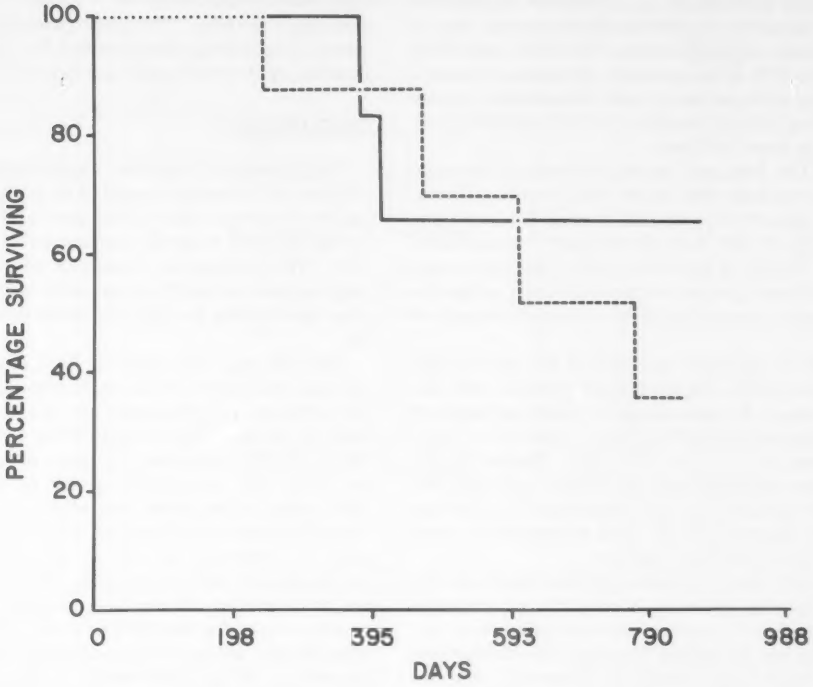

FIGURE 2.—Survival duration for complete responders with localized (dashed line) and extensive (solid line) disease.

for sites of residual disease following induction chemotherapy revealed that eight patients (42%) had primary tumor as the only remaining evidence of measurable disease, and 11 patients (58%) had residual tumor at the primary site and in extrathoracic sites. At the time that progression of disease was noted in 28 patients who have relapsed, the primary tumor was the sole site of progression in six patients (21%). Twelve patients (43%) had progression in the primary tumor and other extrathoracic sites, and ten patients (36%) had progression solely in extrathoracic sites. Evidence of disease progression was generally found at the sites of initial involvement.

Of the 28 extensive-disease patients with disease progression, 24 have died; only three of these patients died solely as the result of the primary tumor. Seven patients died of complications of the primary tumor and extrathoracic metastases, and 11 patients died as a result of distant metastases. Three patients died of infectious complications.

One of these patients with extensive disease presented with left hilar, paratracheal, and lingular masses as well as a pleural effusion and liver metastases. There was resolution of the left hilar and paratracheal lesions with chemotherapy, but the lingular mass and hepatic metastases progressed. At autopsy, SCC was found only in one left hilar node, whereas poorly differentiated adenocarcinoma was present in the lingula with metastases to the lung (left lower lobe), diaphragm, liver (which was virtually replaced by tumor), and portal lymph nodes.

Thirty-eight patients received prophylactic cranial radiotherapy, and intracranial relapse occurred in three of these patients (7.8%). Sixteen patients did not receive prophylactic cranial radiotherapy (12 because of failure to achieve CR or PR with induction therapy and four because of protocol violations), and there were seven intracranial relapses (43.8%) in this group.

Toxicity and Supportive Care

All patients were hospitalized for the first two courses of intensive CAV therapy as described in

our previous publication (5). Moderate nausea and vomiting occurred in most patients on the day of chemotherapy administration. Moderate mucositis occurred in 50% of the patients. Severe esophagitis requiring prolonged parenteral alimentation and/or tube feeding did not develop. No cardiotoxicity was detected in these patients.

During the first and second courses of intensive CAV, the median wbc count nadirs were 121 and 110/mm^3, respectively, and the median platelet count nadirs were 23,000 and 18,000/mm^3, respectively. During a course of intensive CAV, the wbc count was < 1000/mm^3 for an average of 8 days, while the platelet count was < 50,000/mm^3 for an average of 4 days.

There were 82 febrile episodes in the 104 courses of intensive CAV. Patients were treated with antibiotic therapy for an average of 7 days during each course of intensive CAV therapy. Leukocyte transfusions were not used in this study. Platelet transfusions were administered to patients with platelet counts of < 20,000/mm^3 and were used in 43 of 104 courses of intensive CAV. Rbc transfusions were given in 27 of 104 CAV courses.

There were three treatment-related deaths (5.5%) in the series of 54 patients. A patient with localized disease developed massive bilateral pulmonary emboli during her induction therapy. The immediate cause of death was pulmonary infarction, but the patient did have clinical and autopsy evidence of bronchopneumonia, probably related to the thromboemboli as well as to the therapy-induced leukopenia. Two patients with extensive SCC died of pulmonary complications during induction therapy; one of these patients died of a staphylococcal pneumonia that clinically appeared between the two courses of induction CAV when the patient had no granulocytopenia, and the other patient developed progressive respiratory difficulties secondary to pulmonary candidiasis and marked interstitial fibrosis following the second induction CAV course when blood cell counts had returned to normal.

In 21% of the courses of outpatient chemotherapy, there were delays of > 1 week because of hematologic toxicity. The mean percents of planned doses of each drug administered during the first ten courses of chemotherapy are shown in table 3.

DISCUSSION

The intensive induction chemotherapy regimen utilized in this study resulted in a high overall objective response rate of 78% and a CR rate of 26% in this general hospital population of patients with SCC. The therapeutic results of this experimental regimen are certainly comparable with other intensive approaches to SCC reported in the literature (9).

The CR rate and survival data from six widely quoted studies (two multi-institutional and four single-institutional protocols) are listed with our results in table 4. The results from the single-institution studies, including our own, are generally better than the cooperative-group protocols. These differences in response rate and survival probably reflect variations in patient selection and assessment of response as well as the ability of single institutions to deliver intensive therapy in a more uniform fashion. However, our study (which represents one of the most intensive therapeutic efforts without the use of autologous bone marrow transplantation) did not yield better results than the other single-institution protocols.

This study clearly reaffirms the principle that CR must be the goal of therapy for SCC in that complete regression of tumor is the major determinant of survival (figs 1 and 2). Analysis of treatment failures in this series reveals that the primary tumor is a major site of residual disease in partial responders with extensive as well as localized disease and a major area of disease progression in complete and partial responders. However, of the 33 deaths in this study, only three were due solely to the primary tumor. Thus, this lack of complete eradication of the lung primary is only one manifestation of the inability of our systemic chemotherapy to

TABLE 3.—Mean % of planned doses administered/course

	Course									
	1	2	3	4	5	6	7	8	9	10
Cyclophosphamide	95*	94	98	82	77	73	77	65	68	60
Doxorubicin	93	93	98	82	77	56	66	58	56	63
VP-16-213	97	96	98	82	64	75	68	67	68	60
BCNU			91	66	62	78	59	74	75	50
Vincristine			92	83	81	87	78	83	100	100
Methotrexate			95	88	74	81	69	82	98	70
Procarbazine			69	49	49	65	57	59	75	25

*Values = mean % of planned doses given.

TABLE 4.—Summary of treatment results in SCC

Study group*	Ref No.	Therapy†	Stage	No. of patients	CR (%)	Median survival of all patients	1-yr survival
British Medical Research Council	10	Radiotherapy + CMC	Localized	115	—	43 wks	34%
Southwest Oncology Group	11	Radiotherapy + VAC	Localized	108	41	52 wks	50%
			Extensive	250	14	26 wks	—
			Total group	358	22	31 wks	—
Indiana University	12	Radiotherapy + VAC	Localized	19	—	78 wks	26% alive at 26–45+ mos
			Extensive	39	—	36 wks	—
			Total group	58	41	51 wks	—
Radiation Oncology Branch, NCI	13	Radiotherapy + VAC	Localized	29	79	>2 yrs	—
			Extensive	21	48	10.5 mos	—
			Total group	50	66	—	—
NCI-VA Medical Oncology Branch, Washington, DC	3	High-dose CMC	Localized	4	50	13 mos	75%
			Extensive	19	26	8.5 mos	21%
			Total group	23	30	10.5 mos	30%
Baltimore Cancer Research Program, NCI	14	CAV + alternating non-cross-resistant therapy	Localized	37	57	11–12 mos	—
			Extensive	48	29	7.5 mos	—
			Total group	85	41	—	—
Johns Hopkins	This study	CAV ± radiotherapy	Localized	20	40	65 wks	66%
			Extensive	34	18	41 wks	42%
			Total group	54	26	54 wks	51%

*NCI = National Cancer Institute; VA = Veterans Administration.
†CMC = cyclophosphamide, methotrexate, and CCNU; VAC = vincristine, doxorubicin, and cyclophosphamide; CAV = cyclophosphamide, doxorubicin, and VP-16-213.

achieve complete disappearance of tumor in the majority of patients. As noted by other investigators, radiotherapy to the primary tumor (15) and prophylactic cranial radiotherapy (16), when given in adequate doses and schedules, can prevent progression in these specific sites but will not have significant impact on survival until our systemic therapy improves.

It is interesting to note that three of our "treatment failures" in this series had changes in histology documented by biopsy and/or autopsy. We have previously reviewed the problem of changes in morphology in SCC (17). The findings in this therapeutic study re-emphasize the importance of obtaining adequate tissue samples at the time of diagnosis and of considering rebiopsy when a patient relapses or fails to respond to therapy.

The acute toxic effects of the intensive induction chemotherapy regimen were predictable, based on prior experience with high-dose therapy of SCC (3,4). The mortality of this induction therapy was not significantly greater than the toxic effects encountered in less intense regimens (11). Meticulous radiotherapy treatment planning, which minimized the amount of normal lung in the radiation portal and excluded the esophagus from the treatment field, completely avoided the problems with severe esophagitis and pneumonitis noted in other studies of intensive radiotherapy-chemotherapy (13).

Although the acute hematologic toxic effects of this experimental regimen were predictable and manageable, the monthly maintenance chemotherapy superimposed on high-dose induction therapy resulted in bone marrow hypoplasia that delayed 21% of the courses of outpatient chemotherapy. Maximal regression of tumor generally had occurred prior to the interruption and/or attenuation of chemotherapy, so it is not clear what effect this alteration of therapy had on the subsequent course of the tumor. However, it is clear that the doses of drugs in our induction regimen approximate the maximal level that can be safely administered without bone marrow rescue.

The results with this type of highly intensive therapy do not appear superior to the results of less intense regimens, and such high-dose regimens cannot presently be recommended for use outside of experimental protocols. The major challenge in the therapy of SCC remains the achievement of more durable CRs. Although further investigations of new drugs and radiotherapy techniques and sequencing of drugs based on kinetics of the tumor should be pursued, a better understanding of the biology of tumor resistance may be necessary in order to achieve

further gains in therapy. Recent evidence regarding the heterogeneity of a variety of animal and human tumors including SCC (18,19) may have significant implications for the design of therapeutic approaches to this neoplasm.

REFERENCES

1. Greco FA, and Oldham RK. Current concepts in cancer. Small-cell lung cancer. N Engl J Med 301:355–358, 1979.
2. Weiss RB. Small-cell carcinoma of the lung: therapeutic management. Ann Intern Med 88:522–531, 1978.
3. Cohen MH, Creaven PJ, Fossieck BE, et al. Intensive chemotherapy of small cell bronchogenic carcinoma. Cancer Treat Rep 61:349–354, 1977.
4. Ettinger DS, Karp JE, Abeloff MD, et al. Intermittent high-dose cyclophosphamide chemotherapy for small cell carcinoma of the lung. Cancer Treat Rep 62:413–424, 1978.
5. Abeloff MD, Ettinger DS, Khouri NF, et al. Intensive induction therapy for small cell carcinoma of the lung. Cancer Treat Rep 63:519–524, 1979.
6. Abeloff MD, Ettinger DS, and Khouri NF. Intensive induction therapy for small cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO 20:326, 1979.
7. Kaplan EL, and Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
8. Peto R, Pike M, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observations of each patient. I. Introduction and design. Br J Cancer 34:585–612, 1976; II. Analysis and examples. Br J Cancer 35:1–35, 1977.
9. Straus MJ. New developments in the treatment of advanced lung cancer. Am Rev Respir Dis 120:967–971, 1979.
10. Medical Research Council Lung Cancer Working Party. Radiotherapy alone or with chemotherapy in the treatment of small-cell carcinoma of the lung. Br J Cancer 40:1–10, 1979.
11. Livingston RB, Moore TN, Heilbrun L, et al. Small-cell carcinoma of the lung: combined chemotherapy and radiation. Ann Intern Med 88:194–199, 1978.
12. Einhorn LH, Bond WH, Hornback N, et al. Long-term results in combined-modality treatment of small cell carcinoma of the lung. Semin Oncol 5:309–313, 1978.
13. Brereton HD, Kent CH, and Johnson RE. Chemotherapy and radiation therapy for small cell carcinoma of the lung: a remedy for past therapeutic failure. *In* Lung Cancer: Progress in Therapeutic Research (Muggia F, and Rozencweig M, eds). New York, NY, Raven Press, 1979, pp 575–586.
14. Aisner J, Whitacre M, Van Echo DA, et al. Alternating non-cross resistant combination chemotherapy for small cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO 21:453, 1980.
15. Cohen MH, Lichter AS, Bunn PA, et al. Chemotherapy-radiation therapy versus chemotherapy in limited small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 21:449, 1980.
16. Livingston RB. Approaches to the control of central nervous system metastases in patients with small cell carcinoma of the lung. *In* Lung Cancer: Progress in Therapeutic Research (Muggia F, and Rozencweig M, eds). New York, NY, Raven Press, 1979, pp 587–592.
17. Abeloff MD, Eggleston JC, Mendelsohn G, et al. Changes in morphologic and biochemical characteristics of small cell carcinoma of the lung. A clinicopathologic study. Am J Med 66:757–764, 1979.
18. Fidler IJ. Tumor heterogeneity and the biology of cancer invasion and metastases. Cancer Res 38:2651–2660, 1980.
19. Baylin SB, Weisburger WR, Eggleston JC, et al. Variable content of histaminase, L-dopa decarboxylase and calcitonin in small-cell carcinoma of the lung. N Engl J Med 299:105–110, 1978.

Phase I Trial of Chlorozotocin: Attempted Amelioration of Myelotoxicity by Glucose Administration[1]

L. Panasci,* R. Comis, S. Ginsberg, L. Kohn, A. Fitzpatrick, M. Rubert, and T. Scalzo[2]

SUMMARY

Chlorozotocin is a new anticancer agent with the chloroethylnitrosourea cytotoxic moiety attached to the carbon-2 position of glucose. Like other chloroethylnitrosoureas, this agent produces delayed myelotoxicity which is dose-limiting. A phase I trial of chlorozotocin with administration of glucose was done in an attempt to modify the myelotoxicity. The patients received the first course of chlorozotocin (200 mg/m²) in the fasting state and then the second course of chlorozotocin with boluses of a 50% glucose solution. With the second course of chlorozotocin administration, the glucose concentration remained threefold greater than after the first course for at least 1 hour. The plasma half-life and apparent volume of distribution of chlorozotocin were similar following either course. The wbc, neutrophil, and platelet count nadirs after the first course of this agent were not significantly different than the nadirs after the second course. We were unable to modify the myelotoxicity of chlorozotocin with boluses of a 50% glucose solution.

[Cancer Treat Rep 65:647–650, 1981]

The chloroethylnitrosoureas are an important group of antitumor agents with demonstrated activity against lymphoma, melanoma, glioma, small cell carcinoma of the lung, and gastrointestinal malignancies when combined with 5-FU (1–3). However, these agents result in cumulative, delayed myelosuppression that significantly limits their clinical usefulness. Thrombocytopenia and granulocytopenia occur 4–6 weeks after chloroethylnitrosourea administration (2,3). With repeated courses of chloroethylnitrosoureas, there is cumulative injury to the bone marrow. A less myelosuppressive chloroethylnitrosourea might increase the therapeutic index of these agents.

Chlorozotocin is a new chloroethylnitrosourea, with the cytotoxic moiety attached to the carbon-2 position of glucose (fig 1). Chlorozotocin has demonstrated optimal antitumor activity with markedly diminished myelotoxicity in mice as compared to BCNU or CCNU (4). This diminished myelotoxicity in mice appears to be a common property of all nitrosoureas with a glucose moiety in their structure (5,6). Nevertheless, chlorozotocin in phase I–II evaluations in man has myelosuppression as its dose-limiting toxicity (7,8). The mechanism of transport of chlorozotocin into cells is unknown. Glucose is transported into most eukaryotic cells, including wbcs, by facilitated diffusion (9,10). If chlorozotocin is transported into cells by the same transport system as glucose, it might be possible to diminish the myelotoxicity of this agent by competing for the transport site. The following phase I evaluation was designed to determine if infusions of glucose could modify the pharmacokinetics or myelotoxicity of chlorozotocin in man.

PATIENTS AND METHODS

Patients selected for this study had advanced, nonresectable malignancy which was not amenable to standard and conventional therapy including sur-

[1]Received Jan 8, 1980; revised Oct 6, 1980; accepted Oct 29, 1980.

[2]Upstate Medical Center and Veterans Administration Medical Center, Syracuse, NY.

* *Reprint requests to*: Lawrence Panasci, MD, Department of Oncology, Jewish General Hospital, 3755 Chemin de la Cote Ste.-Catherine, Montreal, Quebec, Canada H3T 1E2.

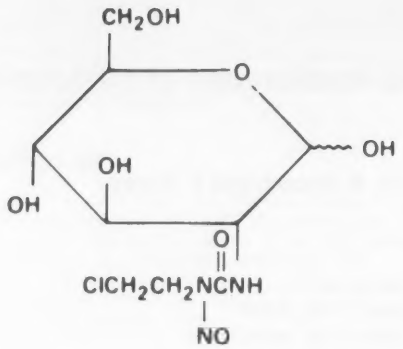

FIGURE 1.—Structure of chlorozotocin.

gery, radiotherapy, or chemotherapy. All patients had a life expectancy > 1 month, a performance status of 0–2 (Cancer and Leukemia Group B performance status criteria), a wbc count ≥ 4000/mm³, a platelet count ≥ 150,000/mm³, and a fasting serum glucose ≤ 105 mg/100 ml. No patient was receiving insulin or oral hypoglycemic agents. All patients received chlorozotocin at a dose of 200 mg/m² by iv bolus administration in the fasting state. The nadir wbc and platelet counts were determined by weekly blood work. If the patient demonstrated a wbc count nadir < 4000/mm³ or a platelet count nadir < 150,000/mm³, he then received the second course of chlorozotocin with glucose infusions 6 weeks after the first course or when the wbc count was ≥ 4000/mm³ and the platelet count was ≥ 150,000/mm³. The second course of chlorozotocin with glucose infusions was given in the following manner. Each patient received 50 g/1.7 m² of iv 50% glucose solution by bolus administration. This bolus of glucose should maintain a serum glucose ≥ 300 mg/100 ml for 15 minutes (11). Chlorozotocin at a dose of 200 mg/m² was administered by iv bolus administration immediately following the glucose. Two additional glucose boluses of 50 g/1.7 m² were given at 15 and 30 minutes after chlorozotocin administration. After the second course of chemotherapy the wbc and platelet count nadirs were compared to those obtained after the first course.

The patients had baseline serum glucose determinations prior to each course of chlorozotocin. The serum glucose levels were repeated at 2.5, 5, 7.5, 10, 15, 30, 45, and 60 minutes after chlorozotocin therapy. Nine patients had plasma chlorozotocin levels determined at the above time points after drug administration without or with glucose. Plasma samples were obtained at bedside by centrifugation of EDTA-anticoagulated whole blood at 500 × *g* for 90 seconds. One milliliter of plasma was added to 0.25 ml of 3 N perchloric acid. This sample was agitated and kept at 0°C for no longer than 90 minutes. The supernatant of the acid-precipitated plasma was obtained by centrifugation at 1000 × *g* for 15 minutes at 4°C. Plasma chlorozotocin levels were then determined by the methodology of Forist (12).

All statistical analysis utilized paired t tests.

RESULTS

Pharmacology

The serum glucose determinations within the first hour of chlorozotocin administration are shown in table 1. In the fasting state, chlorozotocin administration alone did not appear to have any effect on the serum glucose level. The glucose infusions with the second course of chlorozotocin resulted in significant elevations of the serum glucose (at least three times the fasting serum glucose) for a minimum of 1 hour.

A single exponential curve was generated from the plasma chlorozotocin levels determined at 5–30 minutes, with a correlation coefficient of 0.98–0.999. Plasma chlorozotocin levels beyond 30 minutes were generally below the level of sensitivity of the assay. The plasma half-life of chlorozotocin was 12.9 minutes after the administration of chlorozotocin without glucose and 13.5 minutes with glucose. The apparent volumes of distribution were similar when chlorozotocin was administered with or without the glucose infusions (table 2). There was no significant difference in either the half-life or the volume of distribution after glucose administration.

TABLE 1.—Serum glucose levels with chlorozotocin therapy at a dose of 200 mg/m²

Time (mins)	Chlorozotocin alone	Chlorozotocin with glucose*
Baseline	85 ± 11†	92 ± 6
2.5	86 ± 12	414 ± 52
5	85 ± 11	396 ± 65
7.5	86 ± 12	375 ± 55
10	88 ± 11	361 ± 51
15	89 ± 12	343 ± 53
30	92 ± 9	559 ± 96
45	87 ± 11	740 ± 86
60	91 ± 9	632 ± 106

*Chlorozotocin was administered at 200 mg/m² with 50 g/1.7 m² of glucose at 0, 15, and 30 mins after the drug.

†Values = mean ± SD.

TABLE 2.—Pharmacokinetics of chlorozotocin therapy at a dose of 200 mg/m²

	Chlorozotocin alone	Chlorozotocin with glucose	Paired t statistic	Significance
Half-life (mins)	12.9 ± 1.6*	13.5 ± 1.6	0.71	NS†
Apparent volume of distribution (liters)	18.1 ± 5.4	19.7 ± 5.9	0.92	NS

*Values = mean ± SD.
†NS = not significant.

TABLE 3.—Myelosuppression of chlorozotocin

Nadir	Chlorozotocin alone	Chlorozotocin with glucose	Paired t statistic	Significance
Wbc count	2833 ± 864 (40)*	2967 ± 1544 (40)	0.23	NS†
Absolute neutrophil count	1591 ± 821	1851 ± 1074	0.64	NS
Platelet count	94,800 ± 37,500 (23)	79,600 ± 18,200 (25)	1.52	NS

*Values = mean ± SD, with mean Day of nadir after drug administration shown in parentheses.
†NS = not significant.

Myelosuppression

Eight patients completed the study. Two patients had only thrombocytopenia and two patients had only leukopenia after the first drug dose. Four patients developed both thrombocytopenia and leukopenia after the first administration of chlorozotocin. The nadir wbc counts were 2833 and 2967/mm³ after chlorozotocin alone and chlorozotocin administered with the glucose infusions, respectively. The nadirs of the absolute neutrophil counts were similar after either course of chlorozotocin administration (table 3). The nadir platelet counts were 94,800 and 79,600/mm³ with the first and second chlorozotocin therapy, respectively. There are no significant differences among these parameters.

One patient responded to the chemotherapy. A patient with carcinoma of the larynx had a complete response in his cervical node disease after the first dose of chlorozotocin. This response was sustained following the second course with the glucose infusions. The patient relapsed 4 weeks after a third course of chlorozotocin without glucose.

DISCUSSION

The chloroethylnitrosoureas are active antitumor agents, but delayed cumulative myelosuppression limits their clinical usefulness. Chlorozotocin was introduced into clinical trial because of its reduced myelotoxicity in mice (4,6). Unfortunately, its dose-limiting toxicity in man is delayed myelosuppression. Most of the active alkylating agents have myelosuppression as the dose-limiting toxicity, and an amelioration of the myelotoxicity could increase the therapeutic index of these agents.

The results of this investigation demonstrate that infusions of glucose, which elevated the serum glucose to threefold greater than fasting levels and maintained these levels for at least 1 hour, failed to modify the pharmacokinetics or the myelotoxicity of chlorozotocin. It is unlikely that more prolonged infusions of glucose would be more effective, since 1 hour represents four or five plasma half-lives of chlorozotocin.

The results of this study do not, however, rule out the possibility that chlorozotocin is transported into cells by the same carrier system as glucose. It is possible that maximal inhibition of chlorozotocin uptake into cells occurs at 85–100 mg/100 ml of glucose and that further increases in the glucose concentration will have no additional effect. In vitro investigations on the transport of chlorozotocin into L1210 leukemic cells are in progress in our laboratory.

REFERENCES

1. CARTER SK. An overview of the status of the nitrosoureas in other tumors. Cancer Chemother Rep Part 3, vol 4(3):35–46, 1973.

2. WALKER MD. Nitrosoureas in central nervous system tumors. Cancer Chemother Rep Part 3, vol 4(3):21–26, 1973.
3. MOERTEL CG. Therapy of advanced gastrointestinal cancer with the nitrosoureas. Cancer Chemother Rep Part 3, vol 4(3):27–34, 1973.
4. ANDERSON T, MCMENAMIN M, and SCHEIN PS. Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitrosoureidol]-D-glucopyranose, an antitumor agent with modified bone marrow toxicity. Cancer Res 35:761–765, 1975.
5. PANASCI LC, FOX P, and SCHEIN PS. Structure-activity studies of methylnitrosourea antitumor-agents with reduced murine bone marrow toxicity. Cancer Res 37:3321–3328, 1977.
6. PANASCI LC, GREEN D, NAGOURNEY R, ET AL. A structure-activity analysis of chemical and biological parameters of chloroethylnitrosoureas in mice. Cancer Res 37:2615–2618, 1977.
7. HOTH D, WOOLLEY P, GREEN D, ET AL. Phase I studies on chlorozotocin. Clin Pharmacol Ther 23:712–722, 1978.
8. KOVACH JS, MOERTEL CG, SCHUTT AJ, ET AL. A phase I study of chlorozotocin (NSC 178 248). Proc Am Assoc Cancer Res and ASCO 19:408, 1980.
9. MORGAN HE, and WHITFIELD CF. Regulation of sugar transport in eukaryotic cells. Curr Top Membr Transport 4:255–303, 1973.
10. STRAUSS DC, IMHOFF J, and BONVENTRE PF. Membrane transport of amino acids and hexose by guinea pig and mouse phagocytes. J Reticuloendothel Soc 22:403–416, 1977.
11. DYCK DR, and MOORHOUSE JA. A high-dose intravenous glucose tolerance test. J Clin Endocrinol 26:1032–1038, 1966.
12. FORIST AA. Spectrophotometric determination of streptozotocin. Anal Chem 36:1338–1339, 1964.

Iv Dichloromethylene Diphosphonate in Cancer-Associated Hypercalcemia: A Phase I–II Evaluation[1,2]

A. I. Cohen,[3] J. Koeller,[3] T. E. Davis,[3,*] and D. L. Citrin[3,4]

Treatment of the cancer patient with hypercalcemia is often unsatisfactory. Although a saline diuresis augmented by furosemide can produce normocalcemia in most patients (1), the rest require potentially toxic drugs (including high-dose prednisone and mithramycin). Administration of iv fluids clearly requires hospitalization, with increasing cost and morbidity, and this is aggravated by the fact that recurrent episodes of hypercalcemia are not infrequent.

There is a clear need for new nontoxic agents which can be used to treat and prevent hypercalcemia in the cancer patient. Diphosphonates are a class of synthetic organic compounds structurally related to pyrophosphate. They inhibit both bone resorption and mineralization (2,3) and have been used in the treatment of Paget's disease of the bone (4,5). Recently, it has been demonstrated that oral administration of amino hydroxypropylidine diphosphonate lowers serum and urinary calcium and urinary hydroxyproline in a small number of patients with metastatic breast cancer and multiple myeloma (6). Similar results have been obtained with oral use of a related compound, dichloromethylene diphosphonate (Cl_2MDP) (7–9). This communication describes the first clinical evaluation of iv Cl_2MDP in the treatment of hypercalcemia due to malignant disease.

MATERIALS AND METHODS

Fourteen patients with diagnoses of surgically unresectable or metastatic cancer were studied. All patients had significant hypercalcemia, with a serum calcium $\geq$ 11.9 mg/100 ml documented on two occasions. (The normal range for serum calcium in the Clinical Laboratories, University of Wisconsin Clinical Sciences Center, is 8.7–10.1 mg/100 ml.) All patients were clinically stable and provided informed written consent. None had a history of primary hyperparathyroidism or cardiac disease. Any patient who was clinically severely dehydrated and was thought to require iv fluids was excluded from study. Patients who were unable to maintain an oral intake of fluid due to a reduced level of consciousness or confusion were also excluded from study. Any patient with a serum creatinine not greater than 2.5 mg/100 ml or who had received glucocorticoids or mithramycin within 2 weeks or cytotoxic therapy within 1 week was excluded from the study, with one exception.

Cl_2MDP (supplied by Procter & Gamble, Inc) was administered at a dose of 4–20 mg/kg of body weight by 1-hour iv infusion. The first three patients admitted to the study received 4 mg/kg of Cl_2MDP, and dose increments of 4 mg/kg were used for each subsequent group of three patients studied, provided no toxicity was encountered, up to a maximum dose of 20 mg/kg. Cl_2MDP was supplied as a dry, white lyophilized powder. The drug was reconstituted in 125 ml of sterile normal saline immediately prior to infusion. No other iv fluids were given, but patients were allowed to drink freely during the study period. The patients were studied for 72 hours following infusion.

During this time, the patient's clinical condition and serum calcium were closely monitored. Iv fluids were started if the patient's clinical status deteriorated or if there was an increase in serum calcium. Blood was collected immediately before treatment with Cl_2MDP and hourly until 6 hours after infusion. Samples were thereafter obtained at 2-hour intervals until 12 hours and then daily until the conclusion of the study (72 hours). Serum calcium was measured using a Technicon Autoanalyser. Blood was also obtained daily for measurement of serum electrolytes and hematology and chemistry survey. ECG was monitored at 10-minute intervals during infusion of Cl_2MDP.

[1]Received June 18, 1980; revised Sept 15, 1980; accepted Sept 23, 1980.

[2]Approved by the Committee for the Protection of Human Subjects, University of Wisconsin Center for Health Science, Norman Fost, MD, Chairperson. Drs. Davis and Citrin are Junior Faculty Clinical Fellows of the American Cancer Society.

[3]Departments of Human Oncology and Medicine, University of Wisconsin and Wisconsin Clinical Cancer Center, Madison.

[4]*Present address:* Veterans Administration Medical Center and Cancer Center, University of California, San Diego.

**Reprint requests to:* Dr. T. E. Davis, Department of Human Oncology, University of Wisconsin Clinical Cancer Center, Madison, WI 53792.

RESULTS

Fourteen patients (nine males and five females) were studied. Primary diagnosis is shown in table 1. Each patient was studied on one occasion only, except for a single patient (with hypernephroma) who was treated twice at different dose levels. There were 15 treatment trials (three each at doses of 4, 8, 12, 16, and 20 mg of Cl_2MDP/kg of body weight). Pretreatment serum calcium ranged from 11.9 to 15.2 mg/100 ml (median, 12.8).

In this phase I study, no major toxic effects were observed. Iv infusion of Cl_2MDP was not associated with any acute side effects. No patient developed hypocalcemia, tetany, or paresthesias. No ECG abnormalities were detected during or following infusion of the drug. Two patients treated with doses of 4 mg/kg developed transient mild diarrhea following infusion. This required no specific therapy. Two patients had major gastrointestinal bleeding 5 and 30 days following treatment with Cl_2MDP. The first patient had unresectable adrenocortical carcinoma and presented with a serum calcium of 14.4 mg/100 ml. Seventy hours after treatment with Cl_2MDP, his serum calcium was 11.1 mg/100 ml. Three days later he received 60 mg of doxorubicin, and 72 hours later he had a gastrointestinal hemorrhage. Endoscopy revealed two gastric ulcers that appeared to be benign: one in the gastric antrum measuring 1 cm. The second patient had diffuse histiocytic lymphoma with full thickness involvement of the stomach. Twenty-one days after treatment with Cl_2MDP, he received cyclophosphamide (600 mg iv), vincristine (1.2 mg iv), doxorubicin (50 mg iv), and prednisone (100 mg orally × 5). Nine days later, the gastroesophageal junction perforated. The patient had a history of severe bleeding from a gastric ulcer 9 years previously. Neither of these episodes of acute gastrointestinal bleeding are thought to be due to Cl_2MDP.

Ten of 15 patients (66%) responded to infusion of Cl_2MDP with a decrease in serum calcium of > 1 mg/100 ml. Six patients (40%) had an early response, within 13 hours of infusion (median decrease, 1.5 mg/100 ml; range, 1.0–2.4) (table 2). In another four patients (27%), the serum calcium decreased more slowly (mean maximum decrease, 2.0 mg/100 ml; range, 1.1–3.2) (table 3). Two nonresponding patients had an increase in serum calcium over the study period. In one patient, the infusion was discontinued at 20 hours, after an increase in serum calcium from 12.8 to 13.6 mg/100 ml. In the

TABLE 1.—Clinical details

Primary diagnosis	No. of patients	No. of responses	Presumed cause of hypercalcemia
Breast carcinoma	5	2	Bone involvement
Squamous cell carcinoma, lung	2	2	Ectopic PTH*
Non-Hodgkin's lymphoma	2	1	Bone involvement
Oat cell carcinoma, lung	1	0	Bone involvement
Hypernephroma	1	2†	Ectopic PTH
Squamous cell carcinoma, head and neck	1	1	Ectopic PTH
Adrenal carcinoma	1	1	Unknown
Mycosis fungoides	1	1	Unknown
Total	14‡	10	

*PTH = parathyroid hormone.
†1 patient had 2 trials of therapy.
‡9 males and 5 females; median age, 68 yrs (range, 52–79).

TABLE 2.—Results in patients responding early

Dose (mg/kg)	Initial Ca^{++} (mg/100 ml)	Maximum initial decrease		24-hr Ca^{++} (mg/100 ml)	72-hr Ca^{++} (mg/100 ml)
		(mg/100 ml)	(hrs)		
4	14.4	1.5	1	13.0	11.1*
4	12.2	1.1	2	10.7	10.6
8	13.2	2.4	1	11.2	—
12	14.9	1.6	3	13.8	11.5
16	11.9	1.0	8	10.6	9.9†
20	13.7	1.5	13	11.9	10.4

*Received iv fluids.
†48 hrs.

TABLE 3.—Results in late responders

Dose (mg/kg)	Initial Ca^{++} (mg/100 ml)	Maximum initial decrease (mg/100 ml at 12 hrs)	24-hr Ca^{++} (mg/100 ml)	48-hr Ca^{++} (mg/100 ml)	72-hr Ca^{++} (mg/100 ml)
4	12.2	0.9	12.0	10.5	9.8
12	11.9	0.3	10.7	10.0	8.7
20	12.0	0.9	11.1	11.0	10.9
20	14.8	0.6	14.2	13.6	—

other patient, it was discontinued 10 hours after an increase in serum calcium from 12.1 to 13.0 mg/100 ml. Both were given iv saline, which resulted in a decline in the calcium. Another patient whose initial serum calcium of 14.4 mg/100 ml decreased by 1.5 at 1 hour had a secondary increase (to 14.0 mg/100 ml) at 12 hours. Iv fluids were started at this time, and the serum calcium subsequently decreased to 13.0 mg/100 ml at 24 hours and to 11.1 mg/100 ml at 72 hours.

Only three patients, therefore, required iv fluids during the entire study period. Overall, the mean serum calcium level of the group at the conclusion of the study was 11.6 ± 4 mg/100 ml, compared with a prestudy mean of 13.1 ± 3 mg/100 ml ($P < 0.001$, one-tailed Student's t test).

DISCUSSION

Results of this study demonstrate that iv administration of Cl_2MDP can decrease elevated serum calcium due to cancer. The hypocalcemic effect was seen at all dose levels and was not associated with clinically significant toxicity. The calcium-lowering effect was seen in patients who were clinically dehydrated, from whom iv fluids were withheld, thus constituting a significant test of the efficacy of the study drug.

Our results demonstrate that a single iv infusion of the drug can result in significant lowering of serum calcium, and this effect can persist for at least 72 hours. In six of ten responding patients the drug produced a decrease in serum calcium within 12 hours (early response); in the other four, a decrease > 1 mg/100 ml was not observed for 24–48 hours. At this time there is no explanation for the different patterns of response we observed. It is apparently not related to the dose of Cl_2MDP used, as both early and late responses were seen at all dose levels.

The demonstrated efficacy of iv Cl_2MDP, together with the fact that oral administration of the drug for a period of about 7 days can result in a slow decrease of elevated serum calcium to the normal range, raises the possibility of combined iv and oral Cl_2MDP therapy in patients with recalcitrant and/or recurrent cancer-associated hypercalcemia. In this context, the final patient we studied is of interest. He was a 59-year-old man with mycosis fungoides and persistent hypercalcemia. He was treated with Cl_2MDP (20 mg/kg) because of a serum calcium of 13.7 mg/100 ml which had not responded to continuous iv normal saline (250 ml/hour), oral prednisone (60 mg/day), and indomethacin (100 mg/day). Treatment with iv Cl_2MDP resulted in prompt decrease of his serum calcium to 11.9 mg/100 ml at 24 hours and 10.4 mg/100 ml by 72 hours. Thereafter, serum calcium was maintained within normal limits with oral Cl_2MDP (3200 mg/day).

The results of this phase I–II evaluation of iv Cl_2MDP together with recent reports (7–9) of the efficacy and apparent safety of oral Cl_2MDP suggest that this agent may have a useful role in the treatment and prevention of excessive bone destruction and resultant hypercalcemia associated with malignant disease.

REFERENCES

1. SUKI WN, YIUM JJ, VON MINDEN M, ET AL. Acute treatment of hypercalcemia with furosemide. N Engl J Med 283:836–840, 1970.
2. FLEISCH H, RUSSEL RGG, and FRANCIS MD. Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture in vivo. Science 165:1262–1264, 1969.
3. FRANCIS MD. The inhibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates. Calcif Tissue Int 3:151–162, 1969.
4. KHAIRI MRA, ALTMAN RD, DE ROSA GP, ET AL. Sodium etidronate in the treatment of Paget's disease of bone: a study of long term results. Ann Intern Med 87:656–663, 1977.
5. FRIJLINK WB, BIJVOET OLM, VELDE J, ET AL. Treatment of Paget's disease with (3-amino-1-hydroxypropylidine)-1, 1-biphosphonate (APD). Lancet I:799–802, 1979.
6. VAN BREUKELEN FJM, BIJVOET OLM, and VAN OOSTEROM AT. Inhibition of osteolytic bone lesions by (3-amino-hydroxypropylidene)-1, 1-biphosphonate (APD). Lancet I:803–805, 1979.
7. SIRIS ES, SHERMAN WH, and BAQUIRAN DC. Effects of dichloromethylene diphosphonate on skeletal mobilization of calcium in multiple myeloma. N Engl J Med 302:310–315, 1980.
8. DOUGLAS DL, DUCKWORTH T, RUSSEL RGG, ET AL. Effect of dichloromethylene diphosphonate in Paget's disease of bone and in hypercalcemia due to primary hyperparathyroidism or malignant disease. Lancet I:1043–1047, 1980.
9. CHAPUY MC, MEUNIER PJ, ALEXANDRE CM, ET AL. Effects of disodium dichloromethylene diphosphonate on hypercalcemia produced by bone metastases. J Clin Invest 65:1243–1247, 1980.

Phase II Study of AMSA in Lung Cancer[1,2]

Michael K. Samson,[3,*] Roberto J. Fraile,[3] Laurence H. Baker,[3] Glenn Cummings,[3] and Robert W. Talley[4]

SUMMARY

Ninety-one assessable patients with advanced oat cell and non-oat cell carcinoma of the lung were given AMSA on an intermittent every-3-week schedule. Starting doses ranged from 55 to 120 mg/m², depending on the presence and severity of hepatic dysfunction. Three partial responses (two squamous cell carcinomas, one adenocarcinoma) of short duration were documented. The major toxic effect was leukopenia (44%). AMSA does not appear to have sufficient antitumor activity to warrant further investigation in advanced lung cancer.

[Cancer Treat Rep 65:655–658, 1981]

AMSA is one of a series of acridine derivatives synthesized by Cain and Atwell (1) that has reached clinical trials as a potential antitumor agent. AMSA has shown activity against the L1210 and P388 leukemia systems as well as B16 melanoma, spontaneous C3H mammary adenocarcinoma, CD8F, mammary tumors (sc), and transplantable colon tumors No. 26 (intracerebral) and No. 38 (sc) in mice (1). Lack of significant activity has been documented in intracerebrally inoculated L1210, the ependymoblastoma mouse model, MX1 breast xenograft, and CX2 or other colon xenografts in nude mice (1,2). Its mechanism of action is believed to be inhibition of DNA synthesis via intercalation and by an external binding (3). Preclinical pharmacology studies of ^{14}C-AMSA indicated that > 50% of the administered activity was excreted in the bile within 2 hours after drug administration. The bile:plasma ratios of radioactivity were > 400:1 (4). Pharmacokinetic studies done in humans showed a biphasic distribution with an initial rapid-phase half-life of 10–15 minutes and a second slow-phase half-life of 8–9 hours for total AMSA and 3 hours for free AMSA (5).

Phase I clinical studies have indicated the dose-limiting toxic effect to be myelosuppression. Responses have been observed in soft-tissue sarcomas, adenocarcinoma of lung and ovary, malignant melanoma, and acute myeloblastic leukemia (5–7). Because of these encouraging reports, we initiated a phase II clinical trial of AMSA in metastatic lung cancer. Because no clear schedule dependency has been consistently shown in mouse tumor systems, a single, intermittent, every-3-week schedule was selected. In addition, because the preclinical data suggested extensive biliary excretion of the drug, four different dose levels were evaluated depending on the severity of liver dysfunction.

MATERIALS AND METHODS

All patients who were entered in this study had histologic or cytologic evidence of unresectable or metastatic lung cancer no longer amenable to conventional therapeutic measures. The patients were informed as to the investigational nature of the study and gave written consent in accordance with our institutional policy. Other eligibility criteria included no previous chemotherapy within the previous 4 weeks, evidence of hematologic recovery

[1] Received June 18, 1980; revised Sept 10, 1980; accepted Sept 23, 1980.

[2] Supported by contract N01-CM-67105 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Harper-Grace Hospital, Detroit, MI.

[3] Department of Oncology, Wayne State University School of Medicine, Detroit, MI.

[4] Division of Oncology, Department of Medicine, Henry Ford Hospital, Detroit, MI.

**Reprint requests to:* Michael K. Samson, MD, Department of Oncology, Wayne State University School of Medicine, 3990 John R St, Detroit MI 48201.

from previous therapy, presence of measurable disease, a wbc count of ≥ 4000/mm³, a platelet count of ≥ 100,000/mm³, performance status ≥ 40% (Karnofsky scale), and expected survival of at least 6 weeks.

While receiving therapy, patients were evaluated with serial wbc and platelet counts, SMA-17 chemistry panel, chest roentgenograms, tumor measurements, and performance status determination.

Four dose levels were evaluated, based on the degree of liver function impairment and prior therapy: (*a*) 120 mg/m² when liver function was normal and there had been no prior chemotherapy or radiation therapy to major bone marrow sites; (*b*) 100 mg/m² when bilirubin was ≤ 2 mg/100 ml and alkaline phosphatase, SGOT, and/or LDH were elevated but less than twice the normal values; (*c*) 75 mg/m² when bilirubin was > 2 but ≤ 3 mg/100 ml and alkaline phosphatase, SGOT, and/or LDH were at least twice and up to three times the normal values; (*d*) 55 mg/m² when bilirubin was > 3 mg/100 ml and alkaline phosphatase, SGOT, and/or LDH were more than three times the normal values.

Criteria of response were standard. A partial remission was defined as a ≥ 50% decrease in the sum of the products of the largest perpendicular diameters lasting at least 4 weeks in the absence of a significant increase in any one lesion or the appearance of new lesions. Progressive disease was defined as an unequivocal increase (> 25%) in any measured lesion or the appearance of new lesions. Patients were defined as having an "early death" if death occurred within the first 2 weeks of therapy. Signal lesions had to have occurred outside concurrently treated radiation therapy portals. Assessable patients had to have received at least one dose of drug, with a 4-week survival afterward. The calculated dose of AMSA was diluted in 250 ml of 5% dextrose in water and administered in a running iv line over 30–60 minutes. Treatment courses were repeated every 3 weeks, provided there was evidence of recovery from hematologic or nonhematologic toxicity. Dose escalation or de-escalation depended on nadir blood counts and patient tolerance as observed in the previous course of treatment.

PATIENTS

Patient characteristics are summarized in table 1. Of 107 patients entered in this study, 91 were assessable for response. Eleven patients were considered to have had early deaths from tumor-related complications, three were lost to followup, and two died from drug toxicity as will be explained in detail in the Toxicity section.

Of those patients with non-small cell carcinoma, 60% had received no prior chemotherapy. All patients with the diagnosis of small cell carcinoma had received extensive prior chemotherapy, most frequently a combination of cyclophosphamide, doxorubicin, methotrexate, vincristine, and VP-16. In addition, five of these patients also received the phase II agent gallium nitrate prior to entry into this study.

TABLE 1.—Patient characteristics

	Tumor type			
Characteristics*	Squamous cell	Large cell	Adeno-carcinoma	Oat cell
Patients in study	31	15	44	17
Assessable for response	24	12	40	15
Previous chemotherapy	13	3	22	17
Previous radiation therapy	21	8	18	16
Males/females	24/7	9/6	25/19	11/6
Median age (yrs)	59	54	58	57
Median performance status (%)	70	70	75	70
Major sites of metastases				
Local recurrence	4	3	24	2
Visceral	14	4	8	9
Bone	7	6	7	3
Soft tissue	6	2	5	3

*Unless specified otherwise, values = No. of patients.

TABLE 2.—Myelosuppression by dose level

Dose (mg/m^2)	No. of patients	Wbc count (cells × $10^3/mm^3$)			Platelet count (cells × $10^3/mm^3$)		
		Mean	Median	Range	Mean	Median	Range
55	8	6.0	5.9	0.4–13.6	225	197	73–650
75	33	5.0	2.7	0.5–36.3	193	153	45–482
100	13	3.7	3.9	0.9–6.4	210	208	12–419
120	38	4.3	3.3	0.8–20.3	246	224	55–638

RESULTS

Response

Three partial responses were observed: two in patients with squamous cell carcinoma and one in a patient with adenocarcinoma. Response durations were 15, 10, and 9 weeks, respectively. Thus, the overall response rate in non-small cell carcinoma was 4% (three of 76 patients). No objective remissions were noted in patients with small cell carcinoma.

Both responding patients with squamous cell histology had received prior combination chemotherapy consisting of vinblastine, bleomycin, and cisplatin in one patient and cyclophosphamide plus cisplatin followed by dichloromethotrexate in the other.

Toxicity

The major toxic effect was myelosuppression. Table 2 illustrates hematologic toxicity by initial dose level administered. Overall, 44% of the patients developed grade 2 or greater leukopenia ($< 3 \times 10^3$ cells/mm^3), whereas only 3% developed grade 2 or greater thrombocytopenia ($< 7.5 \times 10^4$ cells/mm^3). Collectively, patients with various degrees of hepatic dysfunction who were receiving doses of 55, 75, or 100 mg/m^2 experienced myelosuppression equivalent to that of patients without hepatic impairment who were treated at a dose of 120 mg/m^2 (45% vs 42%, respectively). Generally, maximum recovery occurred by Day 21, allowing subsequent courses of drug to be administered. Nausea and/or vomiting was the major nonhematologic toxic effect (observed in 41% of the patients), while stomatitis, alopecia, and phlebitis at the intravenous site were infrequently seen. One patient developed drug-induced hepatotoxicity, while another individual had an exfoliative dermatitis of the palmar and plantar surfaces that was temporarily related to AMSA administration. No cardiac, renal, or central nervous system toxicity was seen.

Two patients in this study died from toxic reactions. One patient with large cell anaplastic carcinoma, heavily pretreated with chemotherapy and radiation therapy, received a total AMSA dose of 75 mg/m^2 and died on Day 10 with septicemia and grade 4 leukopenia and thrombocytopenia. The second patient, with oat cell carcinoma also pretreated with chemotherapy and radiation therapy, received 55 mg/m^2 of AMSA and died on Day 6 with pneumonia, grade 4 leukopenia, and grade 2 thrombocytopenia.

DISCUSSION

In this phase II study of the acridine derivative AMSA in oat cell and non-oat cell lung cancer, only three partial responses (4%) of short duration were seen. No responses were noted in oat cell cancer. At 95% confidence limits, the predicted true response is in the range of < 1%–11%. Thus, AMSA does not appear to have sufficient antitumor activity to warrant further investigation in advanced lung cancer.

The drug is well-tolerated at the dose levels in this study, is convenient to administer in an outpatient setting, and has predictable toxicity. Based on evidence in rodents of the importance of liver function on metabolism and excretion of AMSA (4), we evaluated four dose levels of AMSA, depending on the degree of liver function impairment. Our results indicated that these levels are reasonable, have manageable toxicity, and may find utility in clinical trials with other tumor types characterized by various degrees of liver function impairment.

REFERENCES

1. CAIN BF, and ATWELL GJ. The experimental antitumor properties of three cogeners of the acridylmethanesulphonanilide (AMSA) series. Eur J Cancer 10:539–549, 1974.
2. OSIEKA R, HOUCHENS DP, GOLDIN A, ET AL. Chemotherapy of human colon cancer xenografts in athymic nude mice. Cancer 40:2640–2650, 1977.
3. WARING MJ. DNA-binding characteristics of acridinylmethanesulphonanilide drugs: comparison with antitumor properties. Eur J Cancer 12:995–1001, 1976.
4. CYSYK RL, SHOEMAKER D, and ADAMSON RH. The pharmacologic disposition of 4′-(9-acridinylamino) methanesulfon-*m*-

anisidide in mice and rats. Drug Metab Dispos 5:579–590, 1977.
5. Van Echo DA, Chiuten DF, Gormley PE, et al. Phase I clinical and pharmacological study of 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide using an intermittent biweekly schedule. Cancer Res 39:3881–3884, 1979.
6. Von Hoff DD, Howser D, Gormley P, et al. Phase I study of methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]-(*m*-AMSA) using a single-dose schedule. Cancer Treat Rep 62:1421–1426, 1978.
7. Legha SS, Gutterman JV, Hall SW, et al. Phase I clinical investigation of 4′-(9-acridinylamino) methanesulfon-*m*-anisidide (NSC-249992), a new acridine derivative. Cancer Res 38:3712–3716, 1978.

Phase II Study of Vindesine in Patients With Metastatic Breast Cancer[1]

Melody A. Cobleigh,* Stephen D. Williams, and Lawrence H. Einhorn[2]

SUMMARY

Twenty-six patients with far-advanced, refractory breast cancer were treated iv with vindesine (DVA) at a dose of 3 mg/m²/week. In 21 evaluable patients there were six partial remissions. Four patients who did not respond after at least four doses of intermittent DVA received continuous-infusion DVA at a dose of 1.5 mg/m²/day × 2 every other week; none responded. Five of the six responders had had disease progression on other vinca alkaloids. Leukopenia and neuromuscular toxicity were dose-limiting.

[Cancer Treat Rep 65:659–663, 1981]

Vindesine (DVA), a new vinca alkaloid, is a derivative of vinblastine (VBL). Doses of 3–4 mg/m²/week have been reported to have activity in breast (1,2), lung (3,4), and esophageal (5) cancers, in adult sarcomas (6), and in melanoma (1). We conducted a phase II investigation of DVA in patients with metastatic breast cancer.

METHODS

Twenty-six patients with biopsy-proven disseminated breast cancer were entered in this trial. Patients were considered evaluable for toxicity if they survived 1 week and for response if they received three doses. Twenty-one patients are evaluable for response and 24 are evaluable for toxicity. Patients inevaluable for toxicity include one who was lost to followup after a single dose and one who was quite debilitated and died 3 days following the first injection (early death). The patients inevaluable for response include these two plus one patient who violated protocol, having received another treatment concurrent with DVA, a second patient who requested no further therapy after one dose, and a third patient who had documented bone marrow involvement with breast cancer, whose platelet count decreased to 45,000/mm³ 1 week after the first injection. Patient characteristics are shown in table 1.

Responses were defined as follows. A complete remission was defined as the complete disappearance of measurable disease. A partial remission was defined as a decrease of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions, in the absence of progression of nonmeasurable disease (bone) for at least 1 month and, in the case of liver involvement, a decrease by one third in the sum of the vertical dimensions of the liver below the costal margins at the midsternal and midclavicular lines. Response duration was calculated from the beginning of chemotherapy until disease progression.

Twenty-five patients received DVA at a dose of 3 mg/m²/week as an iv bolus. Four patients who had received this therapy and had disease progression subsequently received continuous-infusion DVA at a dose of 1.5 mg/m²/day over 24 hours for 2 days every other week via a centrally placed Silastic catheter. Another patient received only continuous-infusion DVA as described above. Therapy was continued until disease progression occurred or until toxicity became dose-limiting. All patients were seen monthly and were evaluated for response. Platelet counts and cbcs were obtained weekly, SMA-12s were done monthly, and other tests were performed as indicated. When patients had persistent neuro-

[1]Received June 26, 1980; revised Oct 6, 1980; accepted Oct 21, 1980.

[2]Department of Medicine, School of Medicine, Indiana University, Indianapolis.

*Reprint requests to: Melody A. Cobleigh, MD, University of Illinois, Abraham Lincoln School of Medicine, PO Box 6998, Chicago, IL 60680.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Menopausal status at diagnosis	
Premenopausal	12
Postmenopausal	12
Unknown	2
Disease-free interval (yrs)	
<1	7
1–3	9
3–5	6
>5	4
Karnofsky performance scale	
>50	19
<50	7
Site of disease	
Bone	16
Nodes and/or soft tissue	11
Visceral	17
Lung	11
Liver	5
Brain	1
Previous hormonal therapy	24
Disease progression on prior combination therapy	25
Disease progression on prior vinca alkaloids	8
Age in yrs	
Mean: 50	
SD: ±10	

muscular toxic effects (paresthesias and/or weakness) or myelosuppression (wbc counts < 3000/mm³) on Day 1 of a new treatment cycle, the interval between treatments was increased to 14 days. If neuromuscular toxic effects became incapacitating (foot drop; profound weakness), therapy was discontinued.

RESULTS

The patients were heavily pretreated (tables 1 and 4). Nearly all patients had had prior hormonal manipulation and all had received prior combination chemotherapy with a median of two combinations; 25 had received doxorubicin, which was usually given in combination with 5-FU and cyclophosphamide. There were six partial remissions (2½, 2½+, 3½+,5+, 6, and 7 months). These occurred in soft tissue and nodes (two patients), lung (three), and liver (one). Because of neuromuscular toxicity, therapy was discontinued in three of these patients despite continued partial remission.

Moderate leukopenia was observed (table 2), with 16 of 24 patients (66%) developing at least one episode of leukopenia < 3000 cells/mm³. Neuromuscular toxicity as manifested by paresthesias and weakness was moderate to severe (table 2); a total of 14 patients experienced such toxicity, eight of whom required a decrease in or discontinuance of treatment.

Miscellaneous reactions included one allergic reaction in a patient who developed urticaria, fever, and rash within 24 hours of drug administration. Myalgia and mild to moderate alopecia were not uncommon.

DISCUSSION

Despite the sensitivity of metastatic breast cancer to combination chemotherapy, this disease is uniformly fatal; thus, the search for new drugs continues. DVA was shown in this study to produce a

TABLE 2.—Toxicity

	No. of patients (%)
Wbc count prior to next planned dose (cells/mm³)	
<1000	2 (8)
1000–1900	6 (25)
2000–2900	8 (35)
3000–3900	4 (17)
Hemoglobin prior to next planned dose (g/100 ml)	
6–7.9	2 (8)
8–9.9	4 (17)
10–12	10 (42)
Platelet count prior to next planned dose (cells/mm³)	
<50,000	1*
50,000–99,000	3 (13)
100,000–150,000	2 (8)
Neurotoxicity†	
Mild	5 (21)
Moderate	3 (13)
Severe	2 (8)
Muscular toxicity†	
Mild	3 (13)
Moderate	4 (17)
Severe	4 (17)
Miscellaneous	
Alopecia	6 (25)
Allergic reaction	1 (4)
Pulmonary edema	1 (4)

*Patient had myelophthisic pancytopenia.

†Mild = required no dose change; moderate = required dose alteration; severe = required discontinuation of drug.

TABLE 3.—Phase II trials of DVA in the treatment of advanced breast cancer

Investigator (ref No.)	No. of—	
	Patients	Responses (%)
Smith et al (1)*	11	1 (9)
Skelley et al (2)	17	3 (18)
Stambaugh et al (9)	8	7 (87)
Jones et al (8)	11	2 (18)
Yap et al (7)	26	2 (7)
Cobleigh et al (present study)	21	6 (29)

*Includes only previously treated patients. 5 of 10 previously untreated patients responded.

response rate of 29% (six responses among 21 patients) in patients having received extensive prior therapy. Only one of the six responders was refractory to doxorubicin (table 4). Three patients had had excellent partial remissions with this drug and had relapsed when they were given other therapy after they had received a dose of 450 mg/m^2; one other patient had received the anthracycline as part of an adjuvant program and relapsed 1 year later; another patient had nonmeasurable disease (bone) but achieved asymptomatic status for 7 months while receiving doxorubicin and vincristine (VCR). The response rate reported here is comparable to most published data (table 3). Five of six responding patients had received vinca alkaloids in the past (two, VBL and three, VCR) and had had disease progression with this therapy. Thus, either there is no cross-resistance among the vinca alkaloids, or previous therapy might have caused further response if given at higher doses or in different schedules. Other authors have reported similar findings (1,8).

We attempted to treat these patients intensively on a weekly schedule; however, toxicity limited our ability to deliver planned doses in 16 of 23 patients (70%). Myelosuppression required dose alterations in eight patients. The incidence of leukopenia is related to the fact that these patients were heavily pretreated. Likewise, neuromuscular toxicity was a significant problem, requiring discontinuance of drug in three patients despite continued response of the patients' cancer, a phenomenon reported by others (5). While only ten patients actually received at least three weekly doses at 3 mg/m^2, three of the six responders fell in the group who could not tolerate this induction schedule and received therapy every other week.

Responses to continuous-infusion DVA have been reported (7) after failure of bolus schedules; consequently, we used continuous-infusion DVA in four patients who had failed intermittent DVA. Although the number is small, no response was seen in the three evaluable patients; this is consistent with the in vitro finding that the lethality of VBL is a function of concentration and time while the efficacy of VCR and DVA is dependent mainly on concentration (10). Another study has shown significant superiority of 5-day continuous-infusion DVA over bolus, but we question the extremely low response rate of 7% (two responses among 26 patients) in the group receiving intermittent therapy. Furthermore, the response rate of 28% (seven responses among 25 patients) in the continuous-infusion group is comparable to that observed in most studies using intermittent DVA (table 3) (7).

We conclude that DVA is an active agent in the treatment of metastatic breast cancer, with a response rate of 29% (confidence limits, 11% and 52% at the $P < 0.05$ level) in heavily pretreated patients (table 4). This response rate may be superior to that observed with VCR as a single agent; with VCR only a 24% response rate was observed in previously untreated patients and less stringent response criteria (25% reduction in tumor volume) were applied (11). In a similar patient population, DVA promises to be highly active, as evidenced by the report of Smith et al (1) in which five of ten untreated patients responded (1). The ultimate role of DVA in combination chemotherapy remains to be defined. The cumulative neuromuscular toxicity observed with weekly doses makes a schedule of 3 mg/m^2 every other week attractive.

TABLE 4.—Prior therapy in responder*

Patient No.	Disease site	Drug schedule	Response and duration
1	Liver	Diethylstilbestrol, 5 mg orally, 3 times daily	PR, 2 yrs
		F, 500 mg/m²; A, 50 mg/m²; and C, 500 mg/m² iv q3wks	PR, received full dose of A
		C, 500 mg/m² over 4 days; M, 30 mg/m², Days 1–8; and F, 500 mg/m², Days 1–8 (orally/mo)	Progressed after 7 mos
2	Lung	Oophorectomy	Progressed after 1 mo
		CMF monthly†	Stable disease, 10 mos; progressed, pleural effusion
		A/VCR†	Resolution of effusion, full dose of A
		Mi/VBL/halotestin†	Disease progression, pleural and bone
3	Lung	FAC (as above) + dibromodulcitol, 135 mg/m² daily × 8 days orally	PR, full dose of A
		CMF (as above)	Progressed after 13 mos
		TMX, 10 mg orally twice a day	Progressed after 8 wks
4	Lung	Oophorectomy	Stable × 5 mos, then progressed
		CMF monthly†	Response duration unknown
		A, 65 mg/m²; and VCR, 2 mg iv q3wks; and TMX, 10 mg orally twice a day†	Initial response unknown but progressed after 6 courses
5	Soft tissue (nodes)	Adjuvant A, 40 mg/m² and C, 600 mg/m² iv q4wks × 6	Relapsed 18 mos later
		Chest wall radiotherapy, 5000 rads per 5½ wks via electron-beam therapy	Progressed during radiotherapy both in and out of field. Hormonal therapy begun 3 mos after radiation therapy was begun
		TMX, 10 mg orally twice a day	Disease progressed after 6 wks
6	Bone, soft tissue (nodes)	Radiation to thoracic spine	Pain relief, 1 mo
		Radiation to left pelvis	Pain relief, 1 mo
		CMF† + radiation to right ribs	Pain relief, 3 mos
		Oophorectomy, A + VCR†	Pain relief, 7 mos. Disease progression in nodes and bone and pleural effusion developed. Since patient was postmenopausal it is doubtful oophorectomy led to pain relief (*see* text)

*A = doxorubicin, F = 5-FU, M = methotrexate, Mi = mitomycin C, C = cyclophosphamide, TMX = tamoxifen.
†Referring institution.

REFERENCES

1. SMITH IE, HEDLEY DW, POWLES TJ, ET AL. Vindesine: a phase II study in the treatment of breast carcinoma, malignant melanoma, and other tumors. Cancer Treat Rep 62: 1427–1433, 1978.
2. SKELLEY M, TORMEY D, ROBINS HI, ET AL. Phase II trial of vindesine in advanced breast cancer. Proc Am Assoc Cancer Res and ASCO 21:172, 1980.
3. DYKE RW, and NELSON RL. Phase I anticancer agents. Vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.
4. FURNAS B, EINHORN LH, and ROHN RJ. A phase II trial of vindesine in non-small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 21:448, 1980.
5. KELSON DP, BAINS M, and CVITKOVIC E. Vindesine in the treatment of esophageal carcinoma: a phase II study. Cancer Treat Rep 63:2019–2021, 1980.
6. MAGILL GD, SORDILLO P, GRALLA R, ET AL. Phase II trials of DVA, AMSA, and BCNU as single agents in adult sarcomas. Proc Am Assoc Cancer Res and ASCO 21:362, 1980.
7. YAP H, BLUMENSCHEIN GR, BODEY GP, ET AL. Continuous five-day infusion vindesine—improvement and therapeutic index in the treatment of refractory breast cancer. Proc Am Assoc Cancer Res and ASCO 21:408, 1980.
8. JONES SE, MILLER TP, DOOR R, ET AL. Phase II trials of vindesine in breast cancer, lymphoma, and other tumors: future directions. *In* Symposium on Vindesine, Washington, DC, 1979.

9. STAMBAUGH JE. Treatment of patients with advanced neoplastic disease, late phase II trial. *In* Symposium on Vindesine, Washington, DC, 1979.
10. GAGG A, ORENGO A, and DREWINKO D. A comparison of the lethal activity of vinca alkaloids: vinblastine, vincristine, and vindesine on cultured human colon carcinoma cells. Proc Am Assoc Cancer Res and ASCO 21:286, 19[illegible]
11. HOLLAND JF, SCHARLAU C, GAILANI S, ET AL. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.

Kinetics of Cisplatin in an Anuric Patient Undergoing Hemofiltration Dialysis[1,2]

Brief Reports and Preliminary Communications

Alain Gouyette,[3,*] Roland Lemoine,[3] Jean-Pierre Adhemar,[4] Dieter Kleinknecht,[4] Ngyen-Khoa Man,[5] Jean-Pierre Droz,[6] and Jean-Pierre Macquet[7,8]

SUMMARY

After in vitro tests, a tracer dose of cisplatin was administered to an anuric cancer patient. Plasma total platinum levels were monitored as a function of time, as was filterable platinum elimination by hemofiltration dialysis. Based on the derived pharmacokinetic parameters, the patient was later given 50-mg doses of cisplatin, once as a single agent, then four times in combination with doxorubicin, VM-26, and cyclophosphamide. Plasma levels could be fitted by a sum of three exponentials after the infusion was stopped. The mean corresponding half-lives are 30 minutes, 6 hours, and 5 days. Depending on the rate of infusion, plasma peaks ranged between 1.1 μg/ml (5.7×10^{-6} M) and 2.27 μg/ml (1.16×10^{-5} M). During the hemofiltration process immediately following the infusion, the elimination rate was first-order, with a mean half-life of 60 minutes. The mean amount eliminated during the first hemofiltration session was 8% of the dose, decreasing to about 3% on Days 2 and 3. A partial tumor response was observed after two treatment courses, evidenced by the relief of abdominal pain and bowel obstruction episodes and a 50% decrease in peritoneal metastases.

[Cancer Treat Rep 65:665–668, 1981]

Cisplatin is now a well-known coordination complex of platinum with antineoplastic efficacy in various types of malignancies (1–4), although the optimal administration in man is still unknown. The most common side effects have included nephrotoxic and ototoxic manifestations, gastrointestinal disturbances, and bone marrow suppression (1). Recent reports indicate that nephrotoxicity can be ameliorated by concomitant use of mannitol or furosemide along with very large infusions of fluid (5) or by using a divided-dose schedule and moderate hydration without use of diuretics (6). But to our knowledge, no anuric patient has been given infusions of cisplatin alone or in combination with other antitumor compounds.

In this preliminary study, we investigated, in vitro, the ability of cisplatin and/or other filterable species to cross a polyacrylonitrile hemofiltration membrane. Then, after administration of cisplatin alone or in combination with doxorubicin, VM-26, and cyclophosphamide, we monitored plasma total platinum levels as well as the elimination of filterable platinum species in an anuric patient undergoing hemofiltration.

CASE REPORT

The patient was 40 years of age when an ovarian adenocarcinoma with peritoneal involvement was discovered in March 1968. She underwent a subtotal hysterectomy with ovariectomy. Surgery was followed by a 1-month pelvic irradiation of 45 Gy and a 1-month total abdominal irradiation of 40 Gy. Complete remission occurred, but bilateral ureteral

[1]Received Feb 19, 1980; revised Aug 15, 1980; accepted Sept 23, 1980.

[2]Supported in part by the Institut Gustave-Roussy (grant FC78A8, contrat de Recherche Clinique) and by the Institut National de la Santé et de la Recherche Médicale (INSERM).

[3]Laboratoire de Pharmacologie Moléculaire associé au Centre National de Recherches Scientifiques (CNRS), and Groupe de Recherche sur la Pharmacologie des Médicaments Anticancéreux de l'INSERM, Institut Gustave-Roussy, Villejuif, France.

[4]Service de Néphrologie et de Réanimation Polyvalente, Unité d'Hémodialyse, Centre Hospitalier Intercommunal, Montreuil, France.

[5]Département de Néphrologie, Hôpital Necker, Paris, France.

[6]Département de Médicine, Institut Gustave-Roussy, Villejuif, France.

[7]Laboratoire de Pharmacologie et Toxicologie Fondamentales (CNRS), Toulouse, France.

[8]We thank J. Escalier for technical assistance and Dr. A. F. LeRoy for help in writing this publication.

*_Reprint requests to:_ Alain Gouyette, PhD, Laboratoire de Chimiothérapie Expérimentale, Institut Gustave-Roussy, 39–53, rue Camille Desmoulins, 94800 Villejuif, France.

fibrosis led to progressive renal failure, and chronic hemodialysis was begun in December 1970. In July 1978, a loss of weight and anorexia occurred, with low-grade fever, abdominal pain, and intermittent bowel obstruction. Peritoneal metastases were palpable and ascites developed. Peritoneal fluid cytology showed tumor cells of the adenocarcinomal type. Liver ultrasonography showed no hepatic involvement.

MATERIALS AND METHODS

Cisplatin was prepared following Dhara's method (7). Its purity was checked by thin-layer chromatography on aluminum sheets coated with silica gel (elution with acetone:0.9% NaCl, 7:3, vol/vol) and by high-performance liquid chromatography using a C_{18} μ-Bondapak column (Waters) and UV detection at 254 nm. The eluent was a 0.9% NaCl solution. In this system, *cis*- and *trans*-diamminedichloroplatinum are separated.

Platinum analysis was performed using a Perkin-Elmer 560 atomic absorption spectrophotometer (AS-1 injector and HGA-500 graphite furnace equipped with pyrocoated tubes), according to a technique described by LeRoy et al (8).

Protein-bound and filterable platinum species were separated through a polyacrylonitrile membrane of the same type as those used for hemofiltration. Immediately after mixing, a 4-ml aliquot of a cisplatin solution (5 and 10 μg/ml) in normal saline or in pooled human plasma was placed in an Amicon stirred ultrafiltration cell (model 8 MC) under a pressure of 70 psi of nitrogen. The volume of ultrafiltrate was 2 ml; each sample was run in duplicate.

The hemofiltration apparatus (Hospal RP 610 type, Rhône-Poulenc, France) was equipped with a polyacrylonitrile membrane (RP-AN 69 type). The substitution fluid contained 140 mEq of Na^+, 1.5 mEq of Mg^{++}, 3.5 mEq of Cl^-, and 40 mEq of CH_3COO^-. The ultrafiltrate flow was 50–60 ml/minute.

After the in vitro studies, the patient was first given an iv infusion of 12 mg of cisplatin in 50 ml of isotonic saline for 60 minutes. One week later she received a 50-mg infusion of cisplatin alone. Later, when she had adequate wbc (> 2500/mm³) and platelet (> 100,000/mm³) counts, she was given a combination of doxorubicin (30 mg iv) on Day 1, VM-26 (50 mg iv) on Day 2, cyclophosphamide (300 mg im) on Days 3 and 4, and cisplatin (50 mg as an iv infusion) on Day 5. Thus, she received a total of six chemotherapy courses (Nov 15 and 22 and Dec 16, 1978, and Jan 22, Mar 12, and May 4, 1979).

Total platinum concentration in plasma as well as filterable platinum species in the ultrafiltrate were monitored following infusions 1, 2, 3, 4, and 6. Blood samples (1 ml) were taken from the arterial blood line of the hemofilter. Aliquots (4 ml) of ultrafiltrate were collected at about 40-minute intervals during the hemofiltration session, which lasted between 4 and 5½ hours.

RESULTS AND DISCUSSION

In Vitro Filtration

We verified that cisplatin and/or some of its "metabolites" could be filtered through a polyacrylonitrile membrane. Recovery of platinum was 90.3% (± 3.7% SEM, $n = 8$) indicating that some platinum had bound either to the membrane or to the wall of the filtration cell. Protein-binding increased with time, as already shown by several authors (9–12).

Plasma Platinum Levels

At first, a tracer dose (12 mg) was given iv, over 1 hour, to calculate the corresponding pharmacokinetic parameters: the peak plasma concentration was 315 ng of Pt/ml (1.6×10^{-6} M). Plasma levels, monitored on 3 consecutive days, could be fitted by a sum of three exponentials, starting from the end of the perfusion. The corresponding α-, β-, and γ-half-lives are 35 minutes, 5.9 hours, and 5.4 days, respectively (fig 1).

Based on these parameters, which were in the same order of magnitude as those reported in the literature (13), we decided to administer 50 mg of cisplatin to this patient 1 week later. Again, plasma levels were triphasic with α-, β-, and γ-half-lives of 20 minutes, 3 hours, and 4.7 days, respectively (fig 1). Furthermore, just prior to the second infusion of cisplatin, the plasma platinum level was found to be 65 ng/ml, in good agreement with the computed value of 69 ng/ml. After this second infusion over 75 minutes, the plasma peak was 1.11 μg/ml (5.7×10^{-6} M), and by the end of the third hemofiltration session (54 hours after the last infusion), the plasma platinum concentration had decreased to 510 ng/ml (2.6×10^{-6} M).

The duration of the infusion was then changed to study its effect on peak plasma levels. After the third (45 minutes), the fourth (90 minutes), and the sixth (60 minutes) infusions, the maximum concentrations were 2.27 μg/ml (1.16×10^{-5} M), 1.20 μg/ml (6.17×10^{-6} M), and 1.51 μg/ml (7.74×10^{-6} M), respectively.

We also measured the residual platinum concentration in plasma just before every infusion was started. These plasma levels were 115 ng/ml (5.9 ×

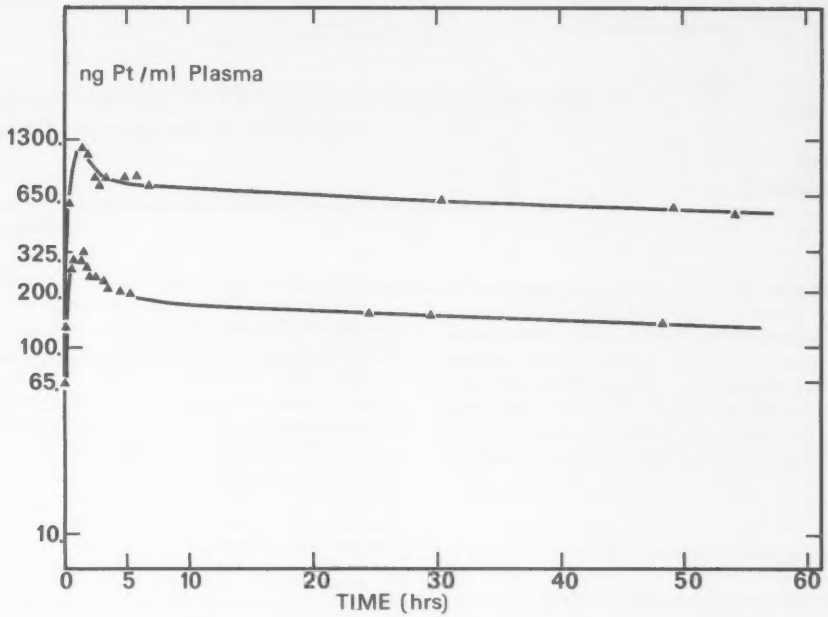

FIGURE 1.—Semilogarithmic plot of plasma total platinum concentration vs time after a 12-mg dose (lower curve) and after a 50-mg dose (upper curve).

10^{-7} M) prior to the third infusion, 138 ng/ml (7.1×10^{-7} M) before the fourth, and 75 ng/ml (4.3×10^{-7} M) before the sixth infusion.

In comparison, by the end of a 1-hour infusion of 70 mg/m², Gormley et al (14) reported a mean value of 30×10^{-6} M total platinum in plasma. Patton et al (15) found a plasma free platinum concentration of 19×10^{-6} M after a 1-hour infusion of 100 mg of cisplatin/m² and 2.6×10^{-6} M after a 6-hour infusion of the same dose.

As our patient's clinical status improved and when blood counts returned to acceptable values, a combination chemotherapy was reinstated. We did not detect any significant changes of the platinum pharmacokinetic parameters when the other antitumor compounds were administered, but we did not determine whether platinum interfered with the protein binding, distribution, and elimination of doxorubicin, VM-26, and cyclophosphamide and/or their respective biotransformation products.

Hemofiltration of Filterable Platinum Species

During hemofiltration, platinum species not yet identified were eliminated into the filtrate. The elimination of platinum as a percent of the administered dose is summarized in table 1.

If we assume that the hemofiltration process is first-order, then the rate of elimination is proportional to the concentration of free platinum in plasma. From the semilogarithmic plot of the rate of elimination versus time, we can determine the decay pattern of the free platinum species in plasma. In fact, this pattern is quite different from that of plasma total platinum levels. We could only detect one phase with a relatively short half-life between 42 and 94 minutes (mean ± SD, 60 ± 20). Patton et al (15) reported a mean value of 43.8 minutes. However, since we had to stop the hemofiltration process after 4 or 5 hours, we could not follow these elimination rate curves for sufficient periods of time; we could only guess the beginning of a slower subsequent phase. Also, the data obtained after the second infusion could be fitted to a sum of two exponentials with half-lives of 13 and 97 minutes, compared to 55 minutes for a single exponential. There is evidence that we could not detect the very early phase. Gormley et al (14) reported such a rapid distribution phase with a half-life of 8–10 minutes, followed by a slower phase with a half-life of 40–45 minutes.

Furthermore, removal of platinum by hemofiltration is less effective than by renal excretion in patients with normal renal function. During the first hemofiltration session, about 8% of the platinum

TABLE 1.—Elimination of filterable platinum species

Infusion No.	% of dose (total ultrafiltrate in liters)		
	Day 1	Day 2	Day 3
1	13.0 (14.4)	5.3 (18.0)	4.1 (15.3)
2	6.5 (16.6)	3.2 (14.7)	3.1 (15.0)
3	9.8 (14.0)		
4	9.4 (18.0)		
6	6.6 (16.0)		

dose is eliminated from the body, compared to 17% excreted by patients with normal renal function within the same period of time (14). However, when the patient underwent hemofiltration on 3 consecutive days, we recovered additional filterable platinum species in the ultrafiltrate (table 1). The mean flow of clearing liquid was about 57 ml/minute and had no significant effect on the amount of platinum eliminated.

Clinical Results

A partial tumor response was evident after two treatment courses. This was evidenced by the relief of abdominal pain and bowel obstruction episodes, a 50% decrease in peritoneal metastases, and a decrease in ascites. Two months later, however, the clinical status of the patient deteriorated again. No hematologic or neurologic toxicity was observed after cisplatin administration. Ototoxicity was also absent in our patient.

In conclusion, this study of the pharmacokinetics of cisplatin is unique in comparison with previous ones (9–18) since the drug was administered to an anuric patient undergoing hemofiltration. This technique probably deserves more detailed studies in animals, especially in the case of combination chemotherapy, but might be useful in patients with renal insufficiency for whom cisplatin is the drug of choice.

REFERENCES

1. ROSENCWEIG M, VON HOFF DD, SLAVIK M, ET AL. *cis*-Diammine-dichloroplatinum (II), a new anticancer drug. Ann Intern Med 86:803–812, 1977.
2. ROSENCWEIG M, VON HOFF DD, PENTA JS, ET AL. Clinical status of *cis*-diammine-dichloroplatinum (II). J Clin Hematol Oncol 7:672–680, 1977.
3. HAYAT M, BAYSSAS M, BRULE G, ET AL. *cis*-Platinum-diammino-dichloro (CPDD) in chemotherapy of cancers. Biochimie 60:935–940, 1978.
4. AMIEL JL, DROZ JP, TURSZ TH, ET AL. Le *cis*-diammine-dichloroplatinum en chimiothérapie. Concours Méd 101:2457–2464, 1979.
5. HAYES DM, CVITKOVIC F, GOLBEY RB, ET AL. High dose *cis*-platinum diammine dichloride amelioration of renal toxicity by mannitol diuresis. Cancer 39:1372–1381, 1977.
6. STARK JJ, and HOWELL SB. Nephrotoxocity of *cis*-platinum (II) dichlorodiammine. Clin Pharmacol Ther 23:461–466, 1978.
7. DHARA SC. A rapid method for the synthesis of *cis*-Pt $(NH_3)_2Cl_2$. Ind J Chem 8:193–194, 1970.
8. LEROY AF, WEHLING ML, SPONSELLER HL, ET AL. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184–191, 1977.
9. LITTERST CL, GRAM TE, DEDRICK RL, ET AL. Distribution and disposition of platinum following intravenous administration of *cis*-diammine-dichloroplatinum (II) (NSC 119875) to dogs. Cancer Res 36:2340–2344, 1977.
10. BANNISTER SJ, STERNSON LA, REPTA AJ, ET AL. Measurement of free-circulating *cis*-dichlorodiammineplatinum (II) in plasma. Clin Chem 23:2258–2262, 1977.
11. MANAKA RC, and WOLF W. Distribution of cis-platin in blood. Chem Biol Interact 22:353–358, 1978.
12. LEROY AF, LUTZ RJ, DEDRICK RL, ET AL. Pharmacokinetic study of *cis*-dichlorodiammineplatinum(II) (DDP) in the beagle dog: thermodynamic and kinetic behavior of DDP in a biologic milieu. Cancer Treat Rep 63:59–71, 1979.
13. SMITH PHS, and TAYLOR DM. Distribution and retention of the antitumor agent ^{195m}Pt-*cis*-dichlorodiammine (II) in man. J Nucl Med 15:349–351, 1974.
14. GORMLEY PE, BULL JM, LEROY AF, ET AL. Kinetics of *cis*-dichloro-diammine platinum. Clin Pharmacol Ther 25:351–357, 1979.
15. PATTON TF, HIMMELSTEIN KJ, BELT R, ET AL. Plasma levels and urinary excretion of filterable platinum species following injection and iv infusion of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 62:1359–1362, 1978.
16. FRICK GA, BALLANTINE R, DRIEVER CW, ET AL. Renal excretion kinetics of high-dose *cis*-dichlorodiammineplatinum(II) administered with hydration and mannitol diuresis. Cancer Treat Rep 63:13–16, 1979.
17. DECONTI RC, TOFTNESS BR, LANGE RC, ET AL. Clinical and pharmacological studies with *cis*-diamminedichloroplatinum (II). Cancer Res 33:1310–1315, 1973.
18. LANGE RC, SPENCER RP, and HARDER HC. The antitumor agent *cis*-Pt $(NH_3)_2Cl_2$: distribution studies and dose calculation for ^{193m}Pt and ^{195m}Pt. J Nucl Med 14:191–195, 1973.

Pharmacokinetics of Hexamethylmelamine and Pentamethylmelamine in Mice[1,2]

Brief Reports and Preliminary Communications

M. Broggini,[3] T. Colombo,[3] M. D'Incalci,[3,*] M. G. Donelli,[3] A. Gescher,[4] and S. Garattini[3]

SUMMARY

Plasma pharmacokinetics of hexamethylmelamine (HMM) and pentamethylmelamine (PMM) were investigated after ip administration of doses of 50 or 100 mg/kg to M5076/73A ovarian tumor-bearing C57BL/6J female mice. The half-life of HMM was 44.5–49 mins; the half-life of PMM was 7.6–8.7 mins. The area under the concentration-versus-time curve (AUC) was also much higher for HMM than for PMM. The AUC for the metabolite N_2,N_2,N_4,N_6-tetramethylmelamine (TMM) was seven to ten times higher than that of the parent compounds. After incubation with mouse liver microsomes and cofactors, the rates of metabolism of HMM and PMM were essentially the same. Plasma protein binding was 94%, 71%, and 50% for HMM, PMM, and TMM, respectively.

[Cancer Treat Rep 65:669–672, 1981]

Hexamethylmelamine (HMM) is an anticancer agent that has shown activity in some experimental and human malignancies (1,2).[5] The compound has to be administered orally because of its poor solubility in physiologic solvents. The variability of oral absorption (3) and the gastrointestinal side effects (1,2), among other factors, led to the testing of more soluble analogs.

Pentamethylmelamine (PMM) is a water-soluble metabolite of HMM that has a similar spectrum of activity in a variety of experimental tumors[6] and therefore was selected for phase I clinical trials currently in progress. Ames et al (4) reported that in rabbits PMM disappears at a faster rate than HMM.

In the present study we report the plasma pharmacokinetics of HMM and PMM in M5076/73A ovarian tumor-bearing mice in which sensitivity to these drugs has been demonstrated,[6] and we describe some investigations on protein binding and metabolism in vitro of these two compounds and of their metabolite N_2,N_2,N_4,N_6-tetramethylmelamine (TMM).

MATERIALS AND METHODS

Animals and drug treatments.—Female C57BL/6J mice (20 ± 2 g body weight) obtained from Charles River Laboratories, Italy, received an sc transplant by trocar of about 2 mm³ of M5076/73A ovarian carcinoma (supplied by Mason Research Institute, DCT-Animal and Human Tumor Bank, Worcester, MA). Twenty days after tumor implantation, animals were treated ip with HMM or PMM at doses of 50 or 100 mg/kg. Both drugs were dissolved in acetone:oil (1:10) solutions. HMM, PMM, and TMM were provided by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Sample collection.—Animals were killed at various times up to 6 hrs after treatment, and at each time the plasma of four animals was collected separately. Urine was collected from mice housed in metabolic cages, at 16, 24, 48, and 90 hrs after drug administration. The urine of four mice was pooled at each collection time for measurement.

[1]Received Mar 14, 1980; revised Sept 17, 1980; accepted Sept 30, 1980.

[2]Supported by CNR Progetto Finalizzato No. 79.00623.96 and by contract N01-CM-97250 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

[4]Visiting scientist at Mario Negri Institute. *Present address:* University of Aston, Gosta Green, Birmingham, BA4YET, United Kingdom.

[5]Legha SS, Slavik M, Livingston RB, et al. Clinical brochure: hexamethylmelamine (NSC-13875). Prepared by the National Cancer Institute, Bethesda, MD, Nov 1974.

**Reprint requests to:* Maurizio D'Incalci, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

[6]Cancer Therapy Evaluation Program. Clinical brochure: pentamethylmelamine (NSC-118742). Prepared by the National Cancer Institute, Bethesda, MD, June 1978.

In vitro metabolic incubation.—Microsomes were obtained from livers of C57BL/6J mice according to Schenkman and Cinti (5). Incubations were carried out with microsomes equivalent to 120 mg/liver/ml incubation medium (Earle's buffer). Drug concentrations were 2 μg/ml (10 μM). Four samples were used for each incubation time with HMM or PMM. The incubation mixture was fortified with 3 mM $MgCl_2$ and cofactors, which generated 1 mM NADPH in a final volume of 2.5 ml. Reactions were started by addition of the drug in 0.1 ml of acetone and were carried out with shaking in the presence of air. No detectable changes in the initial concentrations of HMM or PMM occurred after 60 mins of incubation with boiled and thus inactivated microsomes.

Protein binding studies.—Plasma protein binding was determined by equilibrium dialysis at 37°C. Undiluted plasma (1 ml) containing HMM, PMM, or TMM (1 μg/ml) was dialyzed for 6 hrs against isotonic 0.15 M (pH 7.4) phosphate buffer in Teflon containers separated by a cellophane membrane (Dianorm System). After 6 hrs the drugs were assayed in the liquids on both sides of the membrane. Each protein binding fraction value was the mean of three determinations.

Analytic assay.—HMM, PMM, and TMM from plasma, urine, and microsomes were extracted twice at pH 11.5 with *n*-hexane and once with ethylacetate. A gas chromatographic method with a nitrogen detector, described in detail elsewhere (6), was then applied. Recovery was about 85%. The TMM isomer measured was N_2,N_2,N_4,N_6 as assessed by gas chromatography-mass spectrometry (6).

Pharmacokinetic analysis.—The results were processed using a one-compartment open model after ip administration. The least-squares method was used to determine half-life, using a Hewlett-Packard model 9810 H calculator. The areas under the concentration-versus-time curves (AUC) were calculated by the trapezoidal rule up to 6 hrs.

RESULTS

Figure 1 (A and B) shows the mouse plasma concentration of HMM, PMM, and TMM versus time after ip administration of 50 or 100 mg/kg of HMM. HMM peak levels were reached after 45–60 mins and were 1.4 ± 0.1 and 3.4 ± 0.4 μg/ml after 50 and 100 mg/kg, respectively; HMM plasma half-lives were 44 and 49 mins, respectively; and the $\underrightarrow{AUC}_{360}$ were 129 ± 20 and 393 ± 43 μg/ml × min, respectively. The AUC of PMM formed through *N*-demethylation of HMM were 132 ± 18 and 246 ± 32 μg/ml × min at the two doses, respectively, and the AUC of TMM formed were 1287 ± 190 and 2412 ± 207 μg/ml × min, respectively.

The mouse plasma concentration of PMM and TMM versus time after 50 or 100 mg/kg of PMM are also shown in figure 1 (C and D). Peak levels of PMM were reached in 30 mins and were 2.0 ± 0.5 and 8.5 ± 0.8 μg/ml at the two dose levels, respectively; disappearance was very rapid thereafter and the drug was no longer detectable at 120 mins. The plasma half-lives of PMM were 8 and 9 mins at each dose level, and the $\underrightarrow{AUC}_{360}$ were 66 ± 15 and 352 ± 41 μg/ml × min. After PMM administration the plasma levels of TMM were much higher than those of the parent compound, with AUC of 437 ± 71 and 3237 ± 449 μg/ml × min at the two dose levels.

After HMM at a dose of 100 mg/kg, HMM was not detectable in the urine; the total amounts of PMM and TMM excreted in 90 hrs following treatment were 1.9 μg (0.09% of the initial dose) and 180.8 μg (9.04%), respectively. Within 90 hrs after PMM administration, 1.4 μg of PMM (0.07% of the initial dose) and 85.7 μg of TMM (4.29%) were excreted in the urine. As can be seen in table 1, the disappearance rate of HMM and PMM after in vitro incubation with liver microsomes was found to be equivalent.

Plasma protein binding of HMM, PMM, and TMM was 94% ± 0.6%,71.1% ± 2.9%, and 50% ± 2.7%, respectively.

DISCUSSION

HMM and PMM at the two doses tested appear to have different plasma pharmacokinetics in mice. HMM disappears more slowly, with a half-life of about 46 mins versus 8.5 mins for PMM. These data agree with those reported by Ames et al (4) who found the half-lives of HMM and PMM in the rabbit to be about 67 and 22 mins, respectively.

The faster disappearance of PMM from plasma is not explainable in terms of higher urinary excretion of PMM since in the 90 hrs following PMM administration < 1% of the dose was found in the urine of the treated animals. Another hypothesis is a higher *N*-demethylation rate of PMM than HMM with a consequently faster metabolic clearance. On the basis of our in vitro studies with microsomes, this does not appear likely because HMM and PMM disappear from the metabolism mixture at similar rates (table 1). However, this hypothesis can not be altogether disregarded since the preliminary kinetic analysis of the hepatic microsomal methylmelamine oxidizing system measuring the *N*-hydroxy-methylmetabolites and formaldehyde colorimetrically showed a higher rate of breakdown of the enzyme-melamine

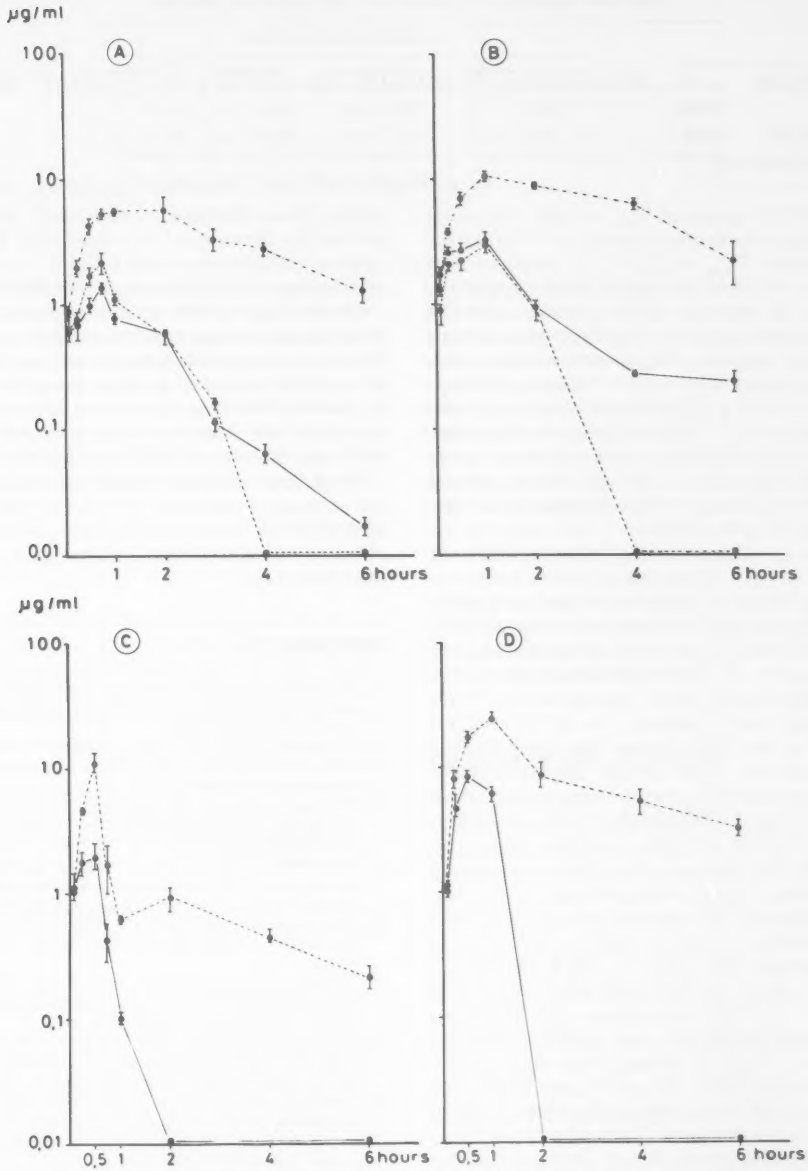

FIGURE 1.—Plasma levels of HMM (——), PMM (- - - -), and TMM (·-·-·) after ip administration of 50 (A) or 100 (B) mg/kg of HMM. Also shown are levels of PMM (——) and TMM (- - - -) after ip administration of 50 (C) or 100 (D) mg/kg of PMM. Both drugs were given to M5076/73A ovarian cancer-bearing C57BL/6J female mice.

TABLE 1.—Percent decrease of the initial 10-μM concentration of HMM and PMM after incubation with mouse liver microsomes and cofactors

	% decrease of drug after—			
	7.5 mins	15 mins	30 mins	60 mins
HMM	95.0 ± 3.3	73.4 ± 1.9	45.6 ± 3.4	35.0 ± 4.7
PMM	88.9 ± 2.7	61.1 ± 4.8	52.8 ± 3.2	31.7 ± 3.6

complex for PMM (apparent V_{max} = 4.20 ± 0.6 nmols HCHO/min/mg microsomal protein, n = 5) than for HMM (apparent V_{max} = 2.6 ± 0.8 nmols/min/mg protein, n = 6) after the insertion of oxygen (C. Brindley and A. Gescher, to be published). At the same time, HMM appeared to exhibit higher affinity to the enzyme (apparent K_M = 90 ± 10 μM) than did PMM (apparent K_M = 230 ± 80 μM). A further hypothesis could be a different distribution of these two compounds since in spite of their structural similarity they have different physicochemical properties (7). We therefore studied the plasma protein binding of the two drugs, which appeared to be 94% for HMM and 71% for PMM.

The different protein binding and the different lipophilicity of the two compounds could in fact influence their tissue distribution. A further hypothesis could also be a higher biliary excretion of PMM. These possibilities are currently being investigated in this laboratory. Another point is that after either HMM or PMM administration, plasma levels of TMM were very high and persistent; the AUC for TMM were seven to ten times higher than those for the parent compounds. Even though the data are not sufficient to evaluate the possible implication of these high and prolonged TMM levels for the pharmacologic effect of HMM or PMM, there are indications that lowering the number of methyls can cause a parallel decrease of antitumor potency in vivo (8). Renal excretion of TMM appears to be an important route of elimination of this metabolite. This was not expected because after ring ^{14}C-HMM administration in mice, Rutty et al (9) identified four major metabolites (trimethylmelamine, dimethylmelamine, monomethylmelamine, and melamine) in urine on the basis of their Rf values. These four metabolites represented 20%, 74%, 4.5%, and 0.61%, respectively, of the total measured radioactivity in the urine samples obtained 72 hrs after treatment, which amounted to about 90% of the total radioactivity administered. The present study shows that TMM appears to be a major urinary metabolite of HMM as well as of PMM in mice. The elimination of TMM corresponded roughly to 9% and 4% of the administered doses of HMM and PMM, respectively. These findings are consistent with those reported by Ames et al (4) who found TMM in the urine of rabbits treated with PMM, in amounts corresponding to 5% of the dose administered.

On the basis of the rapid disappearance of PMM from plasma it might be reasonable to administer PMM in prolonged infusion or several times a day to maintain long-lasting active drug concentrations in plasma. This suggestion is supported by the observation that exposure time is crucial for the in vitro cytotoxicity of HMM and PMM (10).

Work is in progress in this laboratory to study the anticancer and toxic effects of HMM and PMM in M5076/73A tumor-bearing mice treated at different dose schedules based on these pharmacokinetic considerations.

REFERENCES

1. BLUM RH, LIVINGSTON RB, and CARTER SK. Hexamethylmelamine. A new drug with activity in solid tumors. Eur J Cancer 9:195–202, 1973.
2. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine. An evaluation of its role in the therapy of cancer. Cancer 38:27–35, 1976.
3. D'INCALCI M, BOLIS G, MANGIONI C, ET AL. Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62:2117–2119, 1978.
4. AMES MM, POWIS G, KOVACK JS, ET AL. Disposition and metabolism of pentamethylmelamine and hexamethylmelamine in rabbit and human. Cancer Res 39:5016–5021, 1979.
5. SCHENKMAN JB, and CINTI DL. Hepatic mixed function oxidase activity in rapidly prepared microsomes. Life Sci 11:247–257, 1972.
6. D'INCALCI M, MORAZZONI P, and PANTAROTTO C. Gas chromatographic determination of hexamethylmelamine in mouse plasma. Anal Biochem 99:441–449, 1979.
7. CUMBER AJ, and ROSS WCJ. Analogues of hexamethylmelamine: the antineoplastic activity of derivatives with enhanced water solubility. Chem Biol Interact 17:349–357, 1977.
8. LAKE LM, GRUNDEN EE, and JOHNSON BM. Toxicity and antitumour activity of hexamethylmelamine and its N-demethylated metabolites in mice with transplantable tumors. Cancer Res 35:2858–2863, 1975.
9. RUTTY CJ, CONNORS TA, NGUEYEN-HOANG-NAM, ET AL. In vivo studies with hexamethylmelamine. Eur J Cancer 14:713–720, 1978.
10. D'INCALCI M, ERBA E, BALCONI G, ET AL. Time dependence of in vitro cytotoxicity of hexamethylmelamine and its metabolites. Br J Cancer 41:630–635, 1980.

Antitumor Activity of Tuftsin on Murine Melanoma In Vivo[1,2]

Brief Reports and Preliminary Communications

R. Dirk Noyes, George F. Babcock,* and Kenji Nishioka[3,4]

SUMMARY

The effect of tuftsin on malignant melanoma was studied in vivo using a murine model system. It was found that tuftsin was capable of significantly reducing the number of tumor colonies appearing in the lungs of mice following iv administration of tumor cells. Although only a limited number of concentrations of tuftsin were used in our preliminary studies, it appears that tuftsin possesses antitumor activity over a wide range of doses.

[Cancer Treat Rep 65:673–675, 1981]

Substantial evidence has accumulated in recent years to suggest that macrophages and neutrophils are directly cytotoxic to tumor cells (1,2). The mechanism behind the destruction of tumor cells by macrophages or neutrophils is not fully understood. The naturally occurring basic tetrapeptide Thr-Lys-Pro-Arg is a fraction of human IgG that has been shown to stimulate the phagocytic activity of neutrophils and macrophages. This peptide was originally isolated and biochemically defined by investigators at Tufts University, Medford, MA, and subsequently labeled "tuftsin" (3–5).

The physiologic significance of tuftsin was first realized when it was found that people who had had splenectomies and patients with acute myelogenous leukemia, who have high infection rates, have low serum tuftsin levels (6,7). Subsequently, it was found that tuftsin enhanced the in vitro cytotoxic response of human peripheral blood neutrophils and mouse peritoneal macrophages against human and murine tumor cells, respectively (8,9). Tuftsin has also been shown to augment the antigen-specific macrophage-dependent education of T lymphocytes (10). Preliminary in vivo studies indicated that tuftsin increased the survival time of mice bearing L1210 leukemia (9). We are currently analyzing the effect of tuftsin on a murine melanoma model in order to further define its role as a possible immunotherapeutic agent in the treatment of neoplastic disease. The results of our preliminary in vivo studies with tuftsin using this model are reported here.

MATERIALS AND METHODS

The tuftsin used in this study was obtained from Takeda Chemical Industries, Osaka, Japan, and the preparation was synthesized using the liquid-phase procedure (11). The purity of tuftsin was tested using high-performance liquid chromatography and its activity was determined by measuring the enhancement of phagocytosis by human neutrophils (12). Tuftsin preparations purchased from other sources have been found to be of questionable purity and have exhibited variable activity (12). Tuftsin was dissolved in sterile saline and stored at –70°C.

Five-week-old male DBA/2TexAM mice were purchased from Timco Breeding Laboratories, Houston, TX. All studies were performed on mice weighing between 18 and 22 g. Cloudman S-91 melanoma cells (The American Type Culture Collection, Rockville, MD) were maintained in cell culture in Eagle's minimal essential medium (Gibco, Grand Island, NY) supplemented with glutamine (200 mM), gentamicin (50 μg/ml), and 10% fetal bovine serum. Trypsinized tumor cell suspensions were washed in fresh medium and then placed in calcium- and mag-

[1]Received July 7, 1980; revised Nov 13, 1980; accepted Nov 19, 1980.

[2]Supported in part by Public Health Service grants CA-27330 and CA-16672 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Surgery/Surgical Research Laboratory (*R. D. Noyes, G. F. Babcock,* and *K. Nishioka*) and Biochemistry (*K. Nishioka*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]We thank S. Combs and L. Yoshimura for their excellent technical assistance, L. Lerma for help in preparing this manuscript, and Dr. Marvin M. Romsdahl for his guidance and encouragement.

**Reprint requests to:* Dr. George F. Babcock, Department of Surgery/Surgical Research Laboratory, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

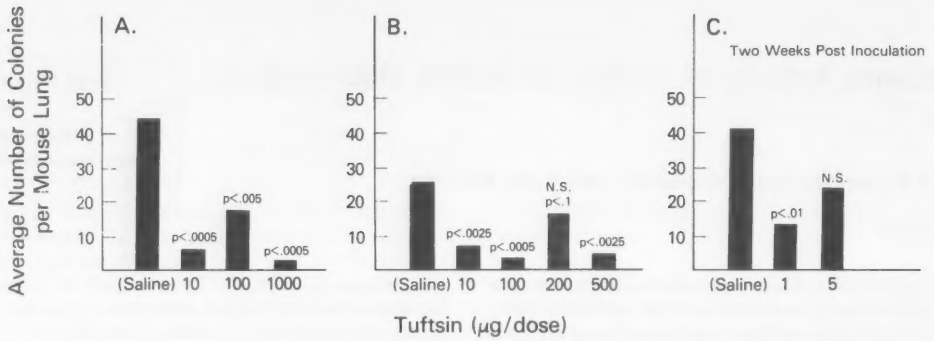

FIGURE 1.—No. of melanoma colonies appearing in the lungs of mice 5 wks after tumor inoculation. Tuftsin therapy started 24 hrs (A and B) or 2 wks (C) after tumor inoculation.

nesium-free Hanks' balanced salt solution at 5°C to prevent cell aggregation.

Mice were divided into control and experimental groups, each consisting of eight mice, and were then inoculated iv with 1×10^5 melanoma cells in a total volume of 0.2 ml as previously described (13). One day after receiving the tumor cells, the mice received ip injections of various doses of tuftsin or 0.9% saline. Treatment schedules varied from a single injection to three injections per week for the duration of the experiment. After the incubation period (5 weeks), all mice were killed and the number of melanoma colonies in the lungs were then counted macroscopically. The number of tumor colonies appearing in the lungs of tuftsin-treated mice was compared with that in those receiving only saline, and the significance was determined using the unpaired Student's t test at 95% confidence limits.

RESULTS

Early experiments using single doses of tuftsin did not appear to have therapeutic effect (data not shown), so a multiple-dose regimen was devised. The data appearing in figures 1A and 1B were obtained using concentrations of tuftsin ranging from 10 to 1000 μg/dose (0.5–50 mg/kg of body weight) given ip three times per week for 5 weeks beginning 1 day after inoculation with tumor cells. The control mice in figure 1A had an average of 44.5 colonies/mouse lung while the mice treated with 1000 μg of tuftsin had only 2.6 colonies/mouse lung. This was a highly significant difference ($P < 0.0005$). Likewise, the mice treated with 10 and 100 μg of tuftsin also had significantly fewer melanoma colonies than the control group. In figure 1B it can be seen that all of the mice treated with tuftsin developed fewer melanoma colonies than the saline-treated mice, with three of the four groups being significantly different (those treated with 10, 100, and 500 μg). The results indicate that tuftsin significantly reduces the average number of melanoma colonies in the lungs of mice over a wide range of concentrations. In our preliminary studies reported here, we have not observed a definite dose response using this multiple-treatment schedule.

An attempt was then made to see if tuftsin still affected tumor growth after a longer period. Since the 10-μg dose used in the previous experiments worked nearly as well as the 1000-μg dose, we decided to lower the dose of tuftsin given to the animals. Experimental groups were given 1 or 5 μg of tuftsin ip three times per week for 3 weeks starting 2 weeks after tumor inoculation, while control animals received only saline. It can be seen in figure 1C that tuftsin was effective in reducing the number of colonies in the lungs at both concentrations, although the group receiving the 5-μg doses was not significantly different from the control group.

DISCUSSION

Malignant melanoma is felt to represent one of the more immunoresponsive tumors. This led to our choice of this tumor to study the antineoplastic properties of the naturally occurring tetrapeptide tuftsin. The Cloudman S-91 murine melanoma model is not a technically difficult system to work with and can yield results in a relatively short time compared to many other in vivo tumor models which rely on death as an end point.

The data presented here suggest that tuftsin sig-

nificantly decreases the number of melanoma colonies in the lungs of mice after iv tumor inoculation. It appears to be effective over a wide range of doses, although we have not observed a dose-response curve using this model. The lack of a dose response was not totally unexpected since only a limited number of doses were tested in this study. Also, each concentration of tuftsin was given many times which could easily have shifted all the doses into the effective range.

It is now known that tuftsin binds to macrophages and neutrophils and in turn stimulates migration and phagocytosis (14–16). These properties have also been shown in vitro using several of the immunopotentiating agents now being used in cancer immunotherapy. Levamisole stimulates the in vitro migration of neutrophils and phagocytic response of monocytes (17,18). BCG-activated macrophages have also increased properties of phagocytosis, pinocytosis, migration, and chemotaxis in in vitro assays (19). Since tuftsin produces similar effects, including the enhancement of antitumor cytotoxicity in vitro, it seemed likely that tuftsin might also be an effective antineoplastic agent. It should also be noted that early experiments with tuftsin using this in vivo model showed an increase in melanoma colonies after single-dose treatments. When a single dose of tuftsin in concentrations of 100 or 200 μg was administered 1 day after tumor inoculation, the average number of tumor colonies increased from just over 50 in the control group to 500 in the treated groups at 5 weeks. This was not unexpected since other immunotherapeutic agents have also been shown to enhance tumor growth (20). Tuftsin was ineffective at any dose tried when only one treatment was given; however, when tuftsin was given in three injections per week, almost all doses were effective. It could be speculated that when a single treatment of tuftsin is given, immunostimulation rather than tumor destruction occurs.

The exact mechanism of action to account for the antineoplastic properties of tuftsin is still unknown, but several hypotheses have emerged. We did not observe any direct cytotoxicity by tuftsin on Cloudman S-91 melanoma cells as evidenced by the similar rates of incorporation of ^{3}H-thymidine between tuftsin-treated and untreated cells. This is in accord with our previously reported results using L1210 leukemia (9). Specific binding sites for tuftsin on macrophages and neutrophils have been identified (21), and we have preliminary data from cell-mediated cytotoxicity in vitro studies that point to the alveolar macrophage as the main effector cell in this tumor model. As larger supplies of pure tuftsin become available, the scope of this project will be expanded.

REFERENCES

1. James K, McBride B, and Stuart A. The Macrophage and Cancer. Edinburgh, Scotland. University of Edinburgh Press, 1977.
2. Klebanoff SJ, and Clark RA. The Neutrophil: Function and Clinical Disorders. New York, NY. North-Holland Publishing Co, 1978.
3. Najjar VA, and Nishioka K. Tuftsin: a physiological phagocytosis stimulating peptide. Nature 228:672–673, 1970.
4. Nishioka K, Constantopoulos A, Satoh PS, et al. The characteristics, isolation and synthesis of the phagocytosis stimulating peptide tuftsin. Biochem Biophys Res Commun 47:172–179, 1972.
5. Nishioka K, Constantopoulos A, Satoh PS, et al. Characteristics and isolation of the phagocytosis-stimulating peptide, tuftsin. Biochem Biophys Acta 310:217–229, 1973.
6. Najjar VA, and Constantopoulos A. A new phagocytosis-stimulating tetrapeptide hormone, tuftsin, and its role in disease. J Reticuloendothel Soc 12:197–215, 1972.
7. Constantopoulos A, Likhite V, Crosby WH, et al. Phagocytic activity of the leukemic cell and its response to the phagocytosis-stimulating tetrapeptide, tuftsin. Cancer Res 22:1230–1234, 1973.
8. Nishioka K. In vitro induction of human granulocyte cytotoxicity by tuftsin. Gan 70:845–846, 1979.
9. ———. Anti-tumor effect of the physiological tetrapeptide, tuftsin. Br J Cancer 29:342–345, 1979.
10. Tzehoval E, Segal S, Stabinsky Y, et al. Tuftsin (an Ig-associated tetrapeptide) triggers the immunogeneic function of macrophages: implications for activation of programmed cells. Proc Natl Acad Sci USA 75:3400–3404, 1978.
11. Nishimura O, and Fujino M. *p*-Methoxybenzensulfonyl as a protecting group of guanido function in peptide synthesis. Chem Pharm Bull (Tokyo) 24:1568–1576, 1976.
12. Amoscato A, Babcock GF, and Nishioka K. A cautionary note regarding contaminants in commercial preparations of the tetrapeptide tuftsin: analysis by high performance liquid chromatography. J Chromatogr 205:179–184, 1981.
13. Takami H, and Nishioka K. Raised polyamines in erythrocytes from melanoma-bearing mice and patients with solid tumours. Br J Cancer 41:751–756, 1980.
14. Nishioka K. Migration enhancement of tuftsin of human mononuclear cells and its effect on the migration inhibition factor test with tumor antigens. Gan 69:569–572, 1978.
15. Nair RMG, Ponce B, and Fudenberg HH. Interactions of radiolabeled tuftsin with human neutrophils. Immunochemistry 15:901–907, 1978.
16. Bar-Shavit Z, Stabinsky Y, Fridkin M, et al. Tuftsin-macrophage interaction: specific binding and augmentation of phagocytosis. J Cell Physiol 100:55–62, 1979.
17. Anderson R, Glover A, Koornhof HJ, et al. In vitro stimulation of neutrophil motility by levamisole: maintenance of cGMP levels in chemotactically stimulated levamisole-treated neutrophils. J Immunol 117:428–432, 1976.
18. Hoebeke J. Influence of tetramisole and its optical isomers on the mononuclear phagocytic system: effect on carbon clearance in mice. J Reticuloendothel Soc 14:317–323, 1973.
19. Poplack DG, Sher NA, Chapara SD, et al. The effect of *Mycobacterium bovis* (bacillus Calmette-Guérin) on macrophage random migration, chemotaxis and pinocytosis. Cancer Res 36:1233–1237, 1976.
20. Hellström KE, and Hellström I. Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv Immunol 18:209–276, 1974.
21. Stabinsky Y, Gottlieb P, Zakuth V, et al. Specific binding sites for the phagocytosis stimulating peptide tuftsin on human polymorphonuclear leukocytes and monocytes. Biochem Biophys Res Commun 83:599–606, 1978.

Venous Thrombosis During Multimodal Treatment of Primary Breast Carcinoma[1,2,3]

Brief Reports and Preliminary Communications

Raymond B. Weiss,* Douglass C. Tormey, James F. Holland, and Vivian E. Weinberg*

SUMMARY

In a cooperative group study of 433 breast cancer patients treated with 2 years of postmastectomy chemotherapy, 22 (5%) developed venous thromboses of various types. Two patients died. None of the patients had demonstrable tumor metastases at the time of the venous thrombosis, although seven had metastases a median of 11 months after the thromboses. Thirteen living patients have not had tumor recurrences. No patient developed thrombosis after the chemotherapy was completed. It is possible that one or more of the chemotherapeutic agents initiated the clotting problems. Thrombophlebitis is a possible problem occurring during adjuvant chemotherapy for breast cancer and requires prompt treatment.

[Cancer Treat Rep 65:677–679, 1981]

Thrombophlebitis has diverse etiologies among which a common one is cancer (1). The pathophysiology of this complication in cancer is thought to be the presence of a hypercoagulable state produced by platelet and other clotting factor alterations (2–4). Thrombophlebitis rarely occurs months to years before clinical diagnosis of a cancer. However, in most instances the patient has a detectable and advanced stage of cancer when the vascular disease occurs (1).

Since 1975, the Cancer and Leukemia Group B has conducted a study comparing different chemotherapeutic regimens for patients with primary breast carcinoma who had at least one axillary lymph node involved with tumor. Despite mastectomy and axillary dissection, many of these patients probably had micrometastases present, and thus chemotherapy was administered. However, they had no detectable cancer after thorough clinical evaluation. A disproportionate frequency of venous thromboses has been seen in these patients. A high frequency of venous clotting abnormalities in patients who have no clinically detectable cancer has not previously been recorded. We report this complication so that it will not be overlooked if suggestive clinical features appear.

MATERIALS AND METHODS

Patients under age 75 at participating institutions of the Cancer and Leukemia Group B who had undergone a radical or modified radical mastectomy within the previous month and had at least one involved axillary node were eligible for this study. Eligible patients had a pretreatment physical examination, chest roentgenograms, bone scans, and mammograms of the remaining breast to exclude the presence of macroscopic metastases. After giving informed consent, patients were randomized to one of three treatment regimens that have been previously described in detail (5): cyclophosphamide, methotrexate, and 5-FU (CMF) plus vincristine and prednisone (regimen A); CMF only (regimen B); and CMF plus methanol extraction residue (MER) of bacillus Calmette-Guérin immunotherapy (regimen C).

Cyclophosphamide was given orally daily and the methotrexate, vincristine, and 5-FU were given iv weekly for the first 6 weeks. The prednisone was

[1]Received Mar 14, 1980; revised Sept 10, 1980; accepted Sept 18, 1980.

[2]Supported by Public Health Service grants CA-R10-16118 and CA-R10-04457 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]This study was conducted by the Cancer and Leukemia Group B at the following and other member institutions: Clinical Investigations Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD (*R. B. Weiss*); Department of Human Oncology, University of Wisconsin, Madison (*D. C. Tormey*); Mt. Sinai School of Medicine, New York, NY (*J. F. Holland, M. Perloff,* and *G. Lesnick*); Cancer and Leukemia Group B Operations Office, Scarsdale, NY (*V. E. Weinberg*); University of Pretoria, South Africa (*G. Falkson*); Rhode Island Hospital, Providence (*M. Gerken*); SUNY-Downstate Medical Center, New York (*S. Rafla*); and Walter Reed Army Medical Center, Washington, DC (*J. Blom*).

**Reprint requests to:* Raymond B. Weiss, MD, Section of Medical Oncology, Walter Reed Army Medical Center, Washington, DC 20012.

given orally daily for 3 weeks, then tapered. The MER was administered every other week intradermally on the trunk for the first 6 weeks. The same drugs were then given for 2 weeks in every four in ten cycles to complete 1 year of treatment. The second year, drugs were administered for 2 in every 8 weeks. After 2 years, the treatment was discontinued and the patients were observed.

RESULTS

There are 433 evaluable patients entered in the comparative chemotherapy study. Of this number, 22 patients have manifested disorders of venous clotting (table 1). Fourteen patients developed thrombophlebitis (superficial and/or deep) of the leg veins, and four of these patients had subsequent pulmonary emboli; all survived. Two patients had pulmonary emboli without recognized antecedent thrombophlebitis; one died. Two patients had thrombosis of mesenteric veins; one died. Four patients had brachial thrombophlebitis.

There was a nearly equal distribution of the venous clotting abnormalities among the three regimens. There were only five premenopausal patients in the group of 22 (23%), although in the entire series, 42% of the patients were premenopausal. Twelve of the 22 patients (54%) had four or more involved axillary nodes at the time of mastectomy, which was similar to the percent (60%) for the whole group of patients.

Thrombosis occurred a median of 3.5 months (range, 1.0–20.5) after mastectomy while the patients were receiving chemotherapy. No instances of thrombosis have occurred in any patient who has completed the 2 years of drug treatment.

All of the patients still living have been observed for tumor recurrence. To date, seven of the 20 patients at risk have had a recurrence (35%), compared to 15% for the entire group of patients in the study. The median time from mastectomy to recurrence for these seven patients was 19.0 months (range, 13.0–31.5), compared to 15.5 months (range, 3.0–38.0) for the entire group. The median time from thrombosis to recurrence was 11.0 months (range, 4.5–25.5). For the 13 living patients without recurrence, the median time from mastectomy is 36.0 months (range, 4.0–43.5), and the median time of followup from thrombosis is 29.0 months (range, 1.0–42.0). Among the 20 living patients, the median disease-free time after mastectomy is 30.0 months.

DISCUSSION

In a study of 433 breast carcinoma patients treated with postmastectomy combination chemotherapy, 5% had manifestations of venous thrombosis. Two patients died; this is a mortality rate of 9.1% for the thrombotic complication or 0.45% for the entire treated group. This incidence of 5% is spread across the three treatment regimens and is much higher than would be expected among ambulatory patients. An untreated comparison control group is not available, but the average incidence of thrombophlebitis in "normal" premenopausal women, for example, has been estimated to be only one to two cases per 1000 women per year (6). There are no similar data available for postmenopausal women.

None of these patients had demonstrable tumor metastases at the time of the clotting problem. However, with a median followup time of 29 months after the thrombotic episode, seven patients have developed tumor metastases. Although there have been reports of venous thrombosis occurring up to 1½ years prior to the appearance of a cancer, this is a rare event (1). The fact that seven patients have had tumor recurrences suggests that occult tumor could have been related to their clotting episode. The recurrences in these seven appeared a median of 19 months after mastectomy and a median of 11 months after the thromboses. Thirteen living patients have not developed recurrences a median of 36 months after the mastectomy. Thus, an etiology other than the presence of occult tumor metastases must be postulated for most of the patients. The likelihood of another etiology is reinforced by the absence of thromboses in the postchemotherapy followup period, despite the fact that some patients no longer receiving chemotherapy are still destined to relapse and thus have occult tumor present. This lack of thromboses in the postchemotherapy period

TABLE 1.—Disorders of venous clotting

Thrombotic episodes by regimen	No. of patients
Regimen A (9 of 143 patients)	
Thrombophlebitis of legs only	5
Thrombophlebitis and pulmonary embolism	1
Pulmonary embolism only	1 (1 death)
Mesenteric venous thrombosis	2 (1 death)
Regimen B (5 of 144 patients)	
Thrombophlebitis of legs only	4
Thrombophlebitis and pulmonary embolism	1
Regimen C (8 of 146 patients)	
Thrombophlebitis of legs only	1
Thrombophlebitis and pulmonary embolism	2
Pulmonary embolism only	1
Thrombophlebitis of arms only	4
Total	22

also diminishes the possibility that the 22 instances of thromboses is a coincidental finding in any population of postmastectomy patients.

It is possible that the chemotherapeutic agents themselves stimulated clotting. Since there was a nearly equal distribution of thromboses among the three treatment regimens, the three agents vincristine, prednisone, and MER cannot be implicated since they were not administered to all patients. Chemotherapeutic agents have been shown to affect clotting in vitro (7,8), but in a manner that enhanced fibrinolysis or inhibited platelet aggregation rather than initiated clotting. Neither cyclophosphamide, methotrexate, nor 5-FU is known to induce clotting, either in vitro or in vivo. If the chemotherapy initiated the clotting episodes in this series of patients, it is puzzling that this phenomenon has not been reported in other large groups of patients treated with similar postmastectomy chemotherapy (9–12). Perhaps instances of thrombosis have occurred, but the possible relationship to the chemotherapy was not appreciated. It seems doubtful that such a problem would be unique to our study, especially since it occurred in 5% of the patients.

There was a disproportion of postmenopausal patients among those with clotting episodes. Whether this fact has any bearing on the etiology of the venous clotting is conjectural at this time.

We report this problem to draw attention to what can be a lethal condition in patients who may be cured of their breast carcinoma. Signs and symptoms of thrombophlebitis in patients who are receiving postmastectomy chemotherapy warrant prompt treatment. Thrombotic complications should not detract, however, from the major benefits of postmastectomy chemotherapy since patients have had not only their disease-free interval but also their overall survival improved (12–14).

REFERENCES

1. Lieberman JS, Borrero J, Urdaneta E, et al. Thrombophlebitis and cancer. JAMA 177:542–545, 1961.
2. Levin M, and Conley CL. Thrombocytosis associated with malignant disease. Arch Intern Med 114:497–500, 1964.
3. Davis RB, Theologides A, and Kennedy BJ. Comparative studies of blood coagulation and platelet aggregation in patients with cancer and nonmalignant diseases. Ann Intern Med 71:67–80, 1969.
4. Miller SP, Sanchez-Avalos J, Stefanski T, et al. Coagulation disorders in cancer. I. Clinical and laboratory studies. Cancer 20:1452–1465, 1967.
5. Weiss R, Tormey D, Glidewell O, et al. Evaluation of postoperative chemoimmunotherapy in mammary carcinoma. Proc Am Assoc Cancer Res and ASCO 20:244, 1979.
6. Drill VA, and Calhoun DW. Oral contraceptives and thromboembolic disease. JAMA 206:77–84, 1968.
7. Komp DM, Lyles RL, Jr, Boyd TH, et al. The effect of cancer chemotherapeutic agents on fibrin formation and stabilization in vitro. Pediatr Res 8:75–81, 1974.
8. Klener P, Kubisz P, and Suranova J. Influence of cytotoxic drugs on platelet functions and coagulation in vitro. Thromb Haemost 37:53–61, 1977.
9. Fisher B, Glass A, Redmond C, et al. L-phenylalanine mustard (L-PAM) in the management of primary breast cancer. An update of earlier findings and a comparison with those utilizing L-PAM plus 5-fluorouracil (5-FU). Cancer 39:2883–2903, 1977.
10. Bonadonna G, Rossi A, Valagussa P, et al. The CMF program for operable breast cancer with positive axillary nodes. Updated analysis on the disease-free interval, site of relapse and drug tolerance. Cancer 39:2904–2915, 1977.
11. Buzdar AU, Gutterman JU, Blumenschein GR, et al. Intensive postoperative chemoimmunotherapy for patients with stage II and stage III breast cancer. Cancer 41:1064–1075, 1978.
12. Cooper RG, Holland JF, and Glidewell O. Adjuvant chemotherapy of breast cancer. Cancer 44:793–798, 1979.
13. Bonadonna G, Valagussa P, Rossi A, et al. Are surgical adjuvant trials altering the course of breast cancer? Semin Oncol 5:450–464, 1978.
14. Fisher B, Redmond C, Fisher ER, et al. The contribution of recent NSABP clinical trials of primary breast cancer therapy to an understanding of tumor biology—an overview of findings. Cancer 46:1009–1025, 1980.

Adjuvant Chemotherapy for Medulloblastoma and Ependymoma Using Iv Vincristine, Intrathecal Methotrexate, and Intrathecal Hydrocortisone: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

Jan van Eys,[3,*] Timothy Chen,[3] Terrence Moore,[4] William Cheek,[5] Charles Sexauer,[6] and Kenneth Starling[7]

SUMMARY

In a prospective, randomized, cooperative group trial, the value of iv vincristine and intrathecal methotrexate and hydrocortisone as adjuvant therapy to radiotherapy in children with medulloblastoma and ependymoma was evaluated. The data showed no improvement in the survival of such children when adjuvant therapy was given.

[Cancer Treat Rep 65:681–684, 1981]

Medulloblastoma and ependymoma are common central nervous system tumors in childhood. The usual approach in treatment has been craniospinal axis irradiation, with a boost to the primary site. While 5-year survival rates approaching 40% have been realized, these do not constitute cure rates, since the relapse and death rates appear to continue beyond 5 years. Therefore, adjuvant chemotherapy is being evaluated for its potential for prolongation of disease-free survival. This report summarizes the Southwest Oncology Group (SWOG) experience using iv vincristine and intrathecal methotrexate and hydrocortisone as additional therapy in patients receiving craniospinal irradiation. Parallel studies have been ongoing in the Children's Cancer Study Group (CCSG), using CCNU, vincristine, and prednisone (1), and in the International Society for Pediatric Oncology (SIOP), using CCNU and vincristine (2). To date the data have been confusing, with the early results of the CCSG study showing no advantage of adjuvant therapy, while the SIOP study seems to show a definite benefit. Our study seems to suggest that there is no advantage to adjuvant chemotherapy using the approach that was selected by the SWOG.

MATERIALS AND METHODS

Patient Eligibility

Patients were eligible for randomization on the protocol if there was a biopsy-proven diagnosis of either medulloblastoma or ependymoma. Patients were not eligible if they had received prior therapy, except for glucocorticoids. The randomization was stratified by medulloblastoma and ependymoma and by the presence or absence of a ventriculoperitoneal or atrial shunt. Thirteen patients had shunts: eight in the group that received radiotherapy alone and five in the group that received adjuvant chemotherapy. For patients with medulloblastoma, these numbers were five and three, respectively.

All patients were evaluated by a physical examination, which included a detailed neurologic examination. A cbc was obtained prior to and during the therapy; spinal fluid was also examined if this could be done safely. An objective radiologic measure of the tumor was obtained: a brain scan, an echoencephalogram, a ventriculogram, a pneumoencephalogram, or an arteriogram. During the course of the study (started in June 1974), CT scans of the brain were available at all institutions. That mode of evaluation superseded less informative radiologic approaches.

[1]Received Apr 10, 1980; revised Aug 21, 1980; accepted Sept 23, 1980.

[2]Supported in part by Public Health Service grants CA-12213, CA-03713, CA-16950, CA-12644, CA-03096, CA-25408, CA-20549, CA-16385, CA-26756, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]Medical University of South Carolina, Charleston.

[5]Baylor School of Medicine, Houston, TX.

[6]Oklahoma Children's Memorial Hospital, Oklahoma City.

[7]Texas Children's Hospital, Houston.

**Reprint requests to:* Dr. Jan van Eys (7409), c/o Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, KS 66103.

All patients had surgical exploration, and as much tumor tissue as possible was resected. All patients were treated with radiotherapy as soon as the surgical wound had healed, usually within 10 days. The irradiation was done with megavoltage equipment, using opposing lateral ports to include the entire intracranial volume and the cervical subarachnoid space to the bottom of the C2 vertebra. The tumor dose used for medulloblastoma was midplane, 4000 rads for children ≥ 3 years of age and 3500 rads for children < 3 years old. In both age groups, the posterior fossa was given an additional 1000 rads. The spinal subarachnoid space was irradiated from the junctional area in the cervical region to the level of S2. The tumor dose was 3000 rads for children ≥ 3 years old and 2500 rads for children < 3 years old. The spinal subarachnoid ports were treated concurrently with the lateral whole-brain ports. After each 1000 rads of tumor dose, the junction between the whole-brain ports and the spinal axis port was moved caudally 1.5 cm. For the treatment of ependymoma, the same ports and spinal cord dose were used. However, for the brain, the tumor dose was 3500 rads regardless of the age of the patient, with a 1500-rad boost for children < 3 years of age and a 2000-rad boost for children ≥ 3 years old. Steroids could be used as necessary for symptoms of increased intracranial pressure. However, steroid therapy was to have been stopped by the time of randomization.

Chemotherapy

After completion of radiotherapy, patients were randomized to receive either no chemotherapy or chemotherapy with vincristine, methotrexate, and hydrocortisone. Vincristine was given at a dose of 2.0 mg/m² iv, with the maximum dose of 2.0 mg. Hydrocortisone and methotrexate were both given in doses of 15.0 mg/m² intrathecally; there was no maximum dose. Initially, the therapy was started on the first week after radiation therapy, with all three drugs given weekly for four doses and then every 4 weeks thereafter for 12 doses (total course, 52 weeks). Doses were delayed for toxicity, but they were not decreased. Serious toxicity with myelosuppression was observed in this schedule. Therefore, the schedule was altered to give vincristine as above, for four doses starting the first week after radiation therapy, followed by vincristine and intrathecal methotrexate plus hydrocortisone weekly for four doses. Then the treatment was given every 4 weeks as above.

RESULTS

Sixty-three patients were entered in the study. Two were ineligible because of incorrect diagnoses, five were not evaluable because of major protocol violations (most did not adhere to the radiotherapy protocol), and 12 were inevaluable because of insufficient data. Of the 44 evaluable patients, nine were only partially evaluable: four were lost to followup after therapy was completed, two refused further therapy, and three had incomplete trials due to toxicity. Sixteen of 34 patients with medulloblastoma received chemotherapy and 18 did not. Three of ten patients with ependymoma received chemotherapy and seven did not.

In the whole group, 11 of 19 patients who received chemotherapy died (median survival, 92 weeks), and seven of 25 patients who did not receive chemotherapy died (median survival > 134 weeks; P value for difference = 0.07). However, among medulloblastoma patients only, eight of the 16 who received chemotherapy died, and five of 18 who received no chemotherapy died (median survival, 128 and > 134 weeks, respectively; $P = 0.22$). Thus, no effect on survival could be seen from this chemotherapy for medulloblastoma (fig 1).

Toxicity

Two children had lethal toxic effects. In one patient, the myelosuppression was so severe that sepsis and death occurred. Another patient had life-threatening myelosuppression, but the exact cause of death was not ascertained. No autopsy was performed. In both of these instances the children had received chemotherapy. Granulocytopenia was present in nearly all patients in both groups. Other toxic effects reported in three or fewer children on chemotherapy were thrombocytopenia, anemia, somnolence, peripheral neuropathy, headaches, constipation, paresthesias, and mucositis. Of these, only thrombocytopenia in one patient, somnolence in two patients, and neuropathy in one patient were reported for the radiotherapy arm. However, no accurate tabulation could be made since the degree of reporting was much more complete for the patient cared for by pediatricians (chemotherapy arm) than it was for patients cared for by radiotherapists (radiotherapy only).

DISCUSSION

The drugs used in this study, iv vincristine and intrathecal methotrexate, have both been shown to

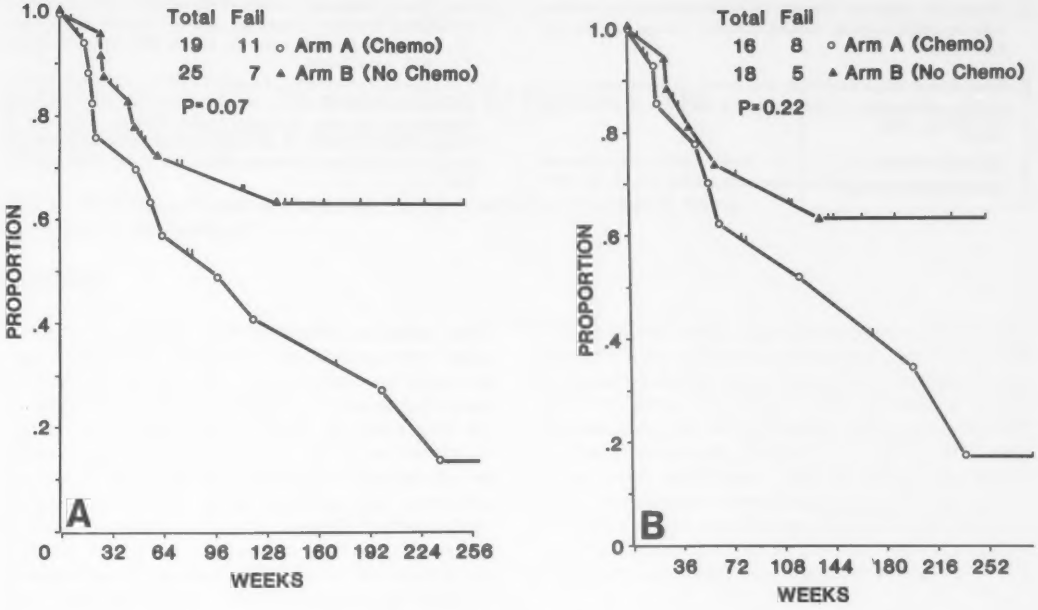

FIGURE 1.—A: survival of all SWOG 7409 patients; B: survival of SWOG 7409 patients with medulloblastoma.

be effective in the treatment of recurrent medulloblastoma (3,4). In fact, recent studies seem to indicate that medulloblastoma, once it is recurrent, is quite responsive to chemotherapy (5). Ependymoma, on the other hand, appears to be far less sensitive to chemotherapy (unpublished SWOG data).

It is therefore surprising that no effect of adjuvant chemotherapy is observed. There are some indications that the effect might appear later in the few patients who have prolonged survival. This seems to be true for the CCSG study (6). However, in our study, no effect on survival was observed. The survival rate among patients receiving radiotherapy only, with the median not reached in nearly 5 years, compares favorably with reports in the literature.

The study was also initially designed to show the effect of adjuvant chemotherapy on remission duration. However, the group receiving chemotherapy received closer and more detailed followup, so they were more likely to be removed from the study at an earlier time than the group receiving no chemotherapy. Therefore, a spurious effect on remission duration was seen which suggested a bias against adjuvant chemotherapy.

The toxic effects observed in this study were severe in those children who received combination chemotherapy and radiotherapy. The same was found in the studies of the CCSG and SIOP. Therefore, alternative approaches to adjuvant therapy must be developed.

The difference in results from the SIOP study is not readily explainable. In our study, there were a substantial number of ineligible or inevaluable patients, and because of the relatively small sample size, it is possible that small differences did not become apparent. The distribution of variables such as diagnosis or shunt placement did not bias one group over another.

Since the overall outcome of the treatment of medulloblastoma is still highly unsatisfactory, further attempts at adjuvant chemotherapy must be made.

REFERENCES

1. EVANS AE, JENKIN RD, ERTEL I, ET AL. Adjuvant chemotherapy for medulloblastoma. Interim report from CCG 942. Proc Am Assoc Cancer Res and ASCO 20:425, 1979.

2. Bloom MJ. Adjuvant therapy for residual disease in children with medulloblastoma. Recent Results Cancer Res 68:412–422, 1978.

3. Rosenstock TG, Evans AE, and Schul L. Response to vincristine of recurrent brain tumors in children. J Neurosurg 45:135–140, 1976.

4. Norrell M, and Wilson CB. Brain tumor chemotherapy with methotrexate given intrathecally. JAMA 201:15–17, 1967.

5. van Eys J. Malignant tumors of the central nervous system. *In* Clinical Pediatric Oncology, 2nd ed (Sutow WW, Vietti TJ, and Fernbach OJ, eds). St. Louis, MO, The CV Mosby Co, 1977, pp 487–508.

6. Evans AE, Anderson J, Chang C, et al. Adjuvant chemotherapy for medulloblastoma and ependymoma. *In* Multidisciplinary Aspects of Brain Tumor Therapy (Paoletti P, Walker MD, Butti G, et al, eds). Amsterdam, The Netherlands, Elsevier/North Holland Biomedical Press, 1979, pp 219–222.

Laparotomy in the Re-evaluation of Patients With Advanced Hodgkin's Disease [1,2]

Brief Reports and Preliminary Communications

Nina C. Kostraba, Bruce A. Peterson,* B. J. Kennedy, Theodor B. Grage, and Clara D. Bloomfield [3]

SUMMARY

Twelve patients with advanced nodular sclerosis Hodgkin's disease were extensively reassessed following the completion of planned chemotherapy. The evaluation culminated in an exploratory laparotomy. Eight patients with no evidence of abdominal involvement on noninvasive tests also had no Hodgkin's disease found during laparotomy. Four patients had intra-abdominal Hodgkin's disease suspected prior to laparotomy based on the results of the noninvasive procedures. At laparotomy, benign abnormalities were present in three patients; only one had residual Hodgkin's disease. No patient with a negative laparotomy has developed recurrent abdominal Hodgkin's disease, but two patients developed supradiaphragmatic recurrences. The patient with persistent Hodgkin's disease had his treatment regimen successfully altered. These data suggest that patients with negative preoperative findings do not benefit further from a laparotomy. However, in selected patients with suspicious intra-abdominal findings, the results of laparotomy may alter future management.

[Cancer Treat Rep 65:685–687, 1981]

The prognosis of patients with advanced Hodgkin's disease has improved dramatically since the introduction of combination chemotherapy. Over 60% of all patients achieve a clinical remission and nearly 50% remain continuously free of disease for ≥ 5 years (1–3). Unfortunately, approximately one third of the patients who achieve a complete remission will eventually relapse. The extent of re-evaluation necessary to detect residual tumor, especially within the abdomen, and to determine which patients will relapse is uncertain. Although a variety of newer noninvasive techniques, such as diagnostic ultrasound and computerized tomography, permit a more extensive search for abdominal tumor than was formerly possible, laparotomy remains the only means of directly assessing this anatomic region.

PATIENTS AND METHODS

Twelve patients, all of whom had received combination chemotherapy for the nodular sclerosis subtype of Hodgkin's disease, were included in this study. The patients ranged in age from 24 to 48 years. There were four females and eight males. Other clinical characteristics are indicated in table 1. Nine patients had recurrences of their Hodgkin's disease following initial therapy with irradiation and were then placed on chemotherapy. Three other patients received combination chemotherapy as the primary mode of therapy. Patients were classified for the extent of Hodgkin's disease according to either the Ann Arbor staging criteria (4) or the Ultmann designation (5) for patients with disease recurrence. Ten patients had previously had staging laparotomies.

The combination of cyclophosphamide, vinblastine, procarbazine, and prednisone (CVPP) was used as initial chemotherapy for 11 patients (1). After six or more cycles of CVPP, the same regimen was continued as maintenance chemotherapy at 2–4-month intervals. One patient (No. 10) was treated with CCNU, vinblastine, procarbazine, and prednisone for 12 months (6). The total duration of chemotherapy for all patients ranged from 12 to 81 months (median, 29).

Reassessment was carried out within 2 months of the cessation of chemotherapy. Informed consent

[1]Received Apr 14, 1980; revised Sept 9, 1980; accepted Sept 18, 1980.

[2]Supported by Public Health Service grant CA-19527 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, by the Masonic Hospital Fund, Inc, and by the Minnesota Medical Foundation.

[3]Section of Medical Oncology, Departments of Medicine and Surgery, University of Minnesota Health Sciences Center and the Masonic Cancer Center, Minneapolis.

Reprint requests to: Bruce A. Peterson, MD, University of Minnesota Hospitals, Box 348, Mayo Memorial Bldg, Minneapolis, MN 55455.

TABLE 1.—Results of re-evaluation in 12 patients treated for advanced nodular sclerosis Hodgkin's disease

Patient No.	Initial stage (Ann Arbor classification)	Prior radiation therapy	Disease status prior to chemotherapy	Abdominal disease prior to chemotherapy	Total duration of chemotherapy (mos)	Abnormal preoperative abdominal studies	Operative findings	Disease-free survival after re-evaluation (mos)
1	IVB*	–	IVB	+	81	Ultrasonography	Hodgkin's disease	–†
2	IIIA	–	IIIA	+	30	Ultrasonography	Hyperplastic nodes (lymphogram effect)	37+
3	IB*	+	TDR‡	+	29	Computerized tomography Ultrasonography	Calcified hematoma	28+
4	IIIA	+	LR‡	–	24	Ultrasonography	Hyperplastic nodes (lipophagic granuloma)	28+
5	IIA*	+	TDR	+	52	None	NED§	36+
6	IIIA	–	IIIA	+	28	None	NED	34+
7	IIA*	+	LR,TDR	+	29	None	NED	30+
8	IIA	+	LR	–	47	None	NED	24+
9	IIA	+	TDR	+	31	None	NED	15+
10	IIA*	+	TDR	+	12	None	NED	9+
11	IIA*	+	TDR	+	14	None	NED	11
12	IIA	+	LR	–	22	None	NED	4

*Patients were initially staged without laparotomy.
†Patient is now disease-free 13 mos following the completion of alternative chemotherapy.
‡Ultmann designation (5): LR = local recurrence; TDR = transdiaphragmatic recurrence.
§NED = no evidence of disease.

was obtained in writing from each patient. The evaluation included a thorough physical examination, bilateral iliac crest bone marrow biopsies, lymphography, and abdominal ultrasonography and/or computerized tomography. Following the completion of these studies all patients underwent a laparotomy, with biopsies taken specifically from sites which appeared abnormal on the noninvasive procedures. The major lymph node regions, both lobes of the liver, and any area which appeared suspicious to the surgeon were also biopsied routinely. Two patients (Nos. 1 and 11) who had not previously undergone staging laparotomy had their spleens removed at the time of reassessment.

RESULTS

Of the 12 patients who underwent reassessment, eight had no evidence of disease following the completion of the noninvasive procedures (table 1). The laparotomy also failed to disclose any Hodgkin's disease in these patients. The other four patients had evidence of retroperitoneal lymphadenopathy in the pelvic and periaortic regions consistent with residual Hodgkin's disease. All four patients had abnormalities during ultrasonography, and in two the abnormal findings were confirmed by either computerized tomography or lymphography. In one patient (No. 3), there were also suspicious areas noted within the liver during ultrasonography. During laparotomy, only one of these patients (No. 1) had residual Hodgkin's disease detected which was within retroperitoneal lymph nodes. The prelaparotomy findings in the other three patients did not represent Hodgkin's disease (table 1).

The 11 patients without detectable Hodgkin's disease had their chemotherapy discontinued. In nine patients, including the three with falsely abnormal findings prior to laparotomy, there has been no disease recurrence after a followup of 9–37 months (median, 28). Two patients (Nos. 11 and 12) relapsed in supradiaphragmatic regions at 4 and 11 months after reassessment, respectively. Patient 1, who was proven to have persistent Hodgkin's disease at re-evaluation, was placed on alternative treatment with CCNU, doxorubicin, bleomycin, vincristine, and prednisone for 22 months of additional chemotherapy. At a second re-evaluation which included another laparotomy, he had no further evidence of

Hodgkin's disease and has had no therapy for 13 months without evidence of disease recurrence.

There were two significant postoperative problems following surgery in these 12 patients. One patient (No. 1) developed a bowel obstruction secondary to adhesions. The other patient (No. 3) developed a pelvic abscess. In both patients, these complications necessitated additional surgery and resulted in a prolongation of their hospitalization.

DISCUSSION

Although advanced Hodgkin's disease can now be cured with chemotherapy in about 50% of patients (2,3), it is likely that, until more precise methods of reassessment are available, some patients will relapse because of ineffective or insufficient therapy. The extent of re-evaluation necessary to predict which patients are likely to relapse or remain in permanent remission is not known. In a study which emphasized the repetition of all previously abnormal tests but did not routinely use laparotomy, residual Hodgkin's disease was detected in 9% of patients immediately following chemotherapy (7). Approximately 15% of the patients who had a negative re-evaluation eventually relapsed. Another study evaluated the routine use of laparotomy in 19 patients with Hodgkin's disease judged to be in a complete clinical remission following chemotherapy (8). These patients had not undergone staging laparotomy at diagnosis. Four patients had Hodgkin's disease detected at surgery in a normal-sized spleen. One of the four patients also had hepatic involvement. All of the patients with residual tumor had Hodgkin's disease of the nodular sclerosis subtype.

Our study included only patients with the nodular sclerosis subtype of Hodgkin's disease. In eight patients who had had normal prelaparotomy evaluations and in three additional patients with findings suspicious for intra-abdominal Hodgkin's disease, residual disease was not found at laparotomy. It is likely that the three patients with suspicious findings would have had their chemotherapy continued without the negative findings of laparotomy. Because of the absence of detectable tumor, treatment was discontinued in all 11 patients. Only one patient had residual Hodgkin's disease discovered at laparotomy and he had abnormalities on the prelaparotomy evaluation. His treatment was altered because of these findings and he subsequently achieved complete remission.

Patients with Hodgkin's disease are likely to relapse if chemotherapy is discontinued because of residual tumor. However, the continuation of chemotherapy in patients who are actually disease-free may lead to unnecessary treatment-related complications. Our data suggest that in patients with no abnormalities detected by the noninvasive tests, additional useful information will not be obtained at laparotomy. None had abdominal Hodgkin's disease detected, but two of our patients, despite completely negative re-evaluation, had supradiaphragmatic recurrences. However, in selected patients where the preoperative findings are inconclusive or lead to the suspicion of residual Hodgkin's disease, surgery may confirm the presence or absence of persistent tumor in the abdomen and contribute to planning future management.

REFERENCES

1. Bloomfield CD, Weiss RB, Fortuny I, et al. Combined chemotherapy with cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) for patients with advanced Hodgkin's disease. An alternative program to MOPP. Cancer 38:42–48, 1976.
2. DeVita VT, Jr, Simon RM, Hubbard SM, et al. Curability of advanced Hodgkin's disease with chemotherapy. Long-term follow-up of MOPP-treated patients at the National Cancer Institute. Ann Intern Med 92:587–595, 1980.
3. Gibbs GE, Peterson BA, Kennedy BJ, et al. Long-term survival of patients with Hodgkin's disease treated with cyclophosphamide, vinblastine, procarbazine and prednisone. Arch Intern Med 141:897–900, 1981.
4. Carbone PP, Kaplan HS, Musshoff K, et al. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 31:1860–1861, 1971.
5. Ultmann JE. Report on workshop: clinical evaluation, staging and prognosis. Natl Cancer Inst Monogr 36:355, 1973.
6. Cooper MR, Pajak TF, Nissen NI, et al. A new effective four-drug combination of CCNU (1-[2-chloroethyl]-3-cyclohexyl-1-nitrosourea) (NSC-79038), vinblastine, prednisone, and procarbazine for the treatment of advanced Hodgkin's disease. Cancer 46:654–662, 1980.
7. Herman TS, and Jones SE. Systematic restaging in Hodgkin's disease. Proc Am Assoc Cancer Res and ASCO 18:97, 1977.
8. Sutcliffe SB, Katz D, Stansfeld AG, et al. Post-treatment laparotomy in the management of Hodgkin's disease. Lancet 2:57–60, 1978.

Treatment of Renal Carcinoma: A Phase III Randomized Trial of Oral Medroxyprogesterone (Provera), Hydroxyurea, and Nafoxidine[1,2]

Brief Reports and Preliminary Communications

Leo L. Stolbach,* Colin B. Begg, Thomas Hall, and John Horton[2]

SUMMARY

Seventy patients with metastatic renal carcinoma were randomized to receive hydroxyurea, nafoxidine, or medroxyprogesterone (Provera) orally. Sixty patients were considered evaluable, with a response rate of 5% for medroxyprogesterone (one complete remission) and hydroxyurea (one partial remission) and a response rate of 16% for nafoxidine (two complete remissions and one partial remission). Differences in response rates and duration of survival were not statistically significant. The major toxicity observed with hydroxyurea was hematologic, and the major toxic effect of nafoxidine was an ichthyosis-like skin rash. Toxicity for medroxyprogesterone was minimal.

[Cancer Treat Rep 65:689–692, 1981]

A number of hormonal and cytotoxic agents have been tested in the management of advanced renal cancer (1,2), but most agents tested to date have shown only limited usefulness. The Eastern Cooperative Oncology Group has designed a series of controlled trials to evaluate hormonal and chemotherapeutic agents used alone and in combination for the treatment of renal carcinoma. One of the early trials tested the effectiveness of medroxyprogesterone (Provera), nafoxidine, and hydroxyurea. The results of that study form the basis for this report.

MATERIALS AND METHODS

Patients with metastatic, recurrent, or unresectable renal carcinoma, not amenable to curative surgery, and who had measurable disease, were randomized to receive one of the three therapies. Patients could have had previous chemotherapy or hormonal therapy, but not with any of the three agents being evaluated in this study. The selection of therapeutic agents was based on whether patients were considered to be "good risk" or "poor risk." Good-risk patients were eligible for randomization to all three agents, and were required to have a BUN < 20 mg/dl, a leukocyte count > 5000/mm^3, and a platelet count > 150,000/mm^3. Patients who did not meet these criteria were classified as poor-risk patients and were randomized only to receive the hormonal agents nafoxidine and medroxyprogesterone. Crossover treatment was randomized and was limited to those patients who entered the initial phase of the study with adequate renal and bone marrow function. Patients in the poor-risk group initially were crossed over to the other hormonal agent after failure of initial treatment.

Distribution of important prognostic variables was similar in the three groups of patients. These included age, performance status, and time from onset of symptoms to entry in the study.

Medroxyprogesterone was given in 100-mg tablets three times a day. Hydroxyurea was given at a dose of 25 mg/kg/day in two divided doses. Nafoxidine was given at a dose of 4 mg/kg daily, to the nearest 30 mg. All drugs were given for 3 months, unless rapid disease progression was observed. Lesions were measured every 4 weeks. A partial response (PR) was defined as a reduction in the sum of the products of the greatest perpendicular di-

[1]Received May 20, 1980; revised Oct 21, 1980; accepted Oct 22, 1980.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Tufts University, School of Medicine, Boston, MA (*L. L. Stolbach*, CA-07190); Sidney Farber Cancer Institute, Boston, MA (*C. B. Begg*, CA-23318); University of Rochester Cancer Center, NY (*T. Hall*, CA-11083); and Albany Medical College, NY (*J. Horton*, CA-06594). Other participating institutions include: University of Alberta Hospital, Edmonton, Canada; Georgetown University School of Medicine, Washington, DC (CA-02824); Jackson Memorial Hospital, Miami, FL; Jefferson Medical College, Philadelphia, PA (CA-14215); Lahey Clinic Foundation, Boston, MA; Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); and Medical College of Virginia, Richmond (CA-10572).

**Reprint requests to:* Leo Stolbach, MD, Department of Public Health, Pondville Hospital, PO Box 111, Walpole, MA 02081.

ameters of all measured lesions of ≥ 50%. A complete response (CR) was defined as the disappearance of all measurable disease. Disease progression was defined as an increase in the sum of the product of the greatest perpendicular diameters of all measured lesions of ≥ 25%, or the appearance of new lesions. Patients classified as having no change did not meet the criteria for disease progression, PR, or CR.

RESULTS

Sixty of 70 patients entered in the initial phase of this study were evaluable (table 1). No data are available for one patient assigned to receive medroxyprogesterone. The nine remaining unevaluable patients are those who had < 2 weeks of treatment either because of early death or because they were lost to followup. The protocol provided for patients to be removed from the study, at the discretion of the senior investigator, because of rapid progression of disease after a 6-week period (instead of 3 months). Nine such patients were removed from the study prior to receiving 3 months of therapy and are included in our analysis (table 1). Two of these patients (one good-risk patient receiving medroxyprogesterone, and one good-risk patient receiving hydroxyurea) entered the crossover phase of the study.

Response

The response rates were 5% for medroxyprogesterone (one CR) and hydroxyurea (one PR) and 16% for nafoxidine (two CRs and one PR) (table 2). The differences in response rates are not statistically significant.

The patient who responded to medroxyprogesterone remained in remission until her death 1 year later. One of the two patients who achieved a CR with nafoxidine maintained this remission for 1 year, and the second patient who sustained a CR remained in remission for 3 years and then survived for an additional 1½ years. However, this latter patient received nafoxidine for a period of only 6 weeks and her medication was discontinued because of severe nausea and vomiting. At that time she was classified as showing no change. Subsequently, she had gradual clearing of several pulmonary lesions and was considered to have a PR 7 months after starting therapy and a CR 10 months after starting therapy. She developed bone metastases 3 years later, but did not have a response when nafoxidine was restarted. She subsequently developed recurrence of her pulmonary lesions and died 5 years after her initial treatment. The patient who had a PR to the nafoxidine had a response lasting 80 days, manifested by reduction in the size of several pulmonary lesions. However, there was no improvement in the patient's general condition despite the apparent regression of tumor, and the patient died 6 months after starting therapy.

The crossover treatment in this study was nafoxidine in eight patients, medroxyprogesterone in six patients, and hydroxyurea in four patients. None of these 18 patients achieved a tumor response during the crossover.

TABLE 1.—Patient groups

	No. of patients		No. of evaluable patients		
Treatment	Total	Unevaluable	Good-risk	Poor-risk	Total
Medroxyprogesterone	25	3	15 (2)*	7 (1)	22
Hydroxyurea	21	2	19 (4)	†	19
Nafoxidine	24	5	14	5 (2)	19
Total	70	10	48	12	60

*Nos. in parentheses = patients treated < 3 mos.
†Not assigned to receive hydroxyurea.

TABLE 2.—Summary of response by treatments

	No. of responses (%)				
Treatment	CR	PR	No change	Disease progression	Totals
Medroxyprogesterone	1 (5)	0 (0)	4 (18)	17 (77)	22
Hydroxyurea	0 (0)	1 (5)	3 (16)	15 (79)	19
Nafoxidine	2 (11)	1 (5)	4 (21)	12 (63)	19

Duration of Survival

The median survival time (in weeks) for the patients in the three treatment groups are as follows: hydroxyurea, 26.3; nafoxidine, 24.2; and medroxyprogesterone, 33.9. These differences are not statistically different. The good-risk patients had a better median survival time than the poor-risk patients, ie, 28 compared to 22.5 weeks.

Toxicity

The toxic effects observed are shown in table 3. There was minimal toxicity with medroxyprogesterone and it is questionable whether the severe bleeding seen in one patient was indeed due to the medroxyprogesterone. The major toxicity observed with hydroxyurea was hematologic, and the major toxic effect of nafoxidine was an ichthyosis-like skin rash which required discontinuation of therapy in at least two patients.

DISCUSSION

The response rate to cytotoxic chemotherapy has been disappointingly low in renal carcinoma, with most reports showing a response rate of ≤ 10%. Our experience with hydroxyurea agrees with these results. Hormonal agents, ie, medroxyprogesterone and testosterone, have been reported by Bloom (1) to result in a response rate of 17% in a review of 228 reported cases. In another review, Hrushesky and Murphy (2) have reported a response rate of only 2% in 415 patients treated with hormones. Our response rates of 5% for medroxyprogesterone and 16% for nafoxidine are not significantly different from the previously reported results for hormonal treatments. In a recent report by Feun et al (3), one PR was seen among 20 evaluable patients treated with nafoxidine. In our series, two of the three patients who responded to nafoxidine showed complete regression of tumor for 1 and 3½ years. However, the patient with the longest regression received only 6 weeks of therapy, and at the time of discontinuation of therapy was considered to have had no change. Her CR occurred several months after the discontinuation of nafoxidine and could represent a spontaneous regression of tumor.

There have been a number of recent reports documenting estrogen receptors in renal carcinoma (4–6). It is possible that if the patients whose tumors are hormone-dependent could be identified, and if only these patients were treated with hormonal manipulation, their response rate might be significantly higher than those previously reported. Further studies with the newer antiestrogen, tamoxifen, may be indicated in the future even though there have been conflicting reports of its efficacy in renal cancer (7,8).

REFERENCES

1. Bloom HJG. Adjuvant therapy for adenocarcinoma of the kidney: present position and prospects. Br J Urol 45:237–257, 1973.

Table 3.—Toxic effects

Toxic effect	No. of patients receiving—		
	Medroxy-progesterone	Hydroxyurea	Nafoxidine
No toxicity	17*	3	10
Vomiting			
Mild	0	3	1
Moderate	1	1	2
Severe	0	1	1
Bleeding			
Mild	0	0	0
Moderate	0	0	1
Severe	1	0	0
Skin			
Mild	0	0	1
Moderate	0	1	4
Severe	0	0	2
Hematologic			
Mild	1	4	1
Moderate	0	6	0
Severe	0	4	0

*No record of toxicity available for 2 patients receiving medroxyprogesterone.

2. Hrushesky WJ, and Murphy GP. Current status of the therapy of advanced renal carcinoma. J Surg Oncol 9:277–288, 1977.
3. Feun LG, Drelichman A, Singhakowinta A, et al. Phase II study of nafoxidine in the therapy for advanced renal carcinoma. Cancer Treat Rep 63:149–150, 1979.
4. Friedman MA, Hoffman PG, and Jones HW. The clinical value of hormone receptor assays in malignant disease. Cancer Treat Rev 5:185–194, 1978.
5. Concolino G, Marocchi A, Conti C, et al. Human renal cell carcinoma as a hormone-dependent tumor. Cancer Res 38:4340–4344, 1978.
6. Bojar H, Dreyfurst R, Balzer K, et al. Oestrogen-binding components in human renal cell carcinoma. J Clin Chem Clin Biochem 14:521–526, 1976.
7. Al-Sarraf M. The clinical trial of tamoxifen in patients with advanced renal cancer: a SWOG study. Proc Am Assoc Cancer Res and ASCO 20:378, 1979.
8. Glick JH, Wein A, Torri S, et al. Phase II study of tamoxifen in patients with advanced renal cell carcinoma. Cancer Treat Rep 64:343–344, 1980.

Ifosfamide: A Weekly Dose Fractionated Schedule in Bronchogenic Carcinoma[1,2,3]

Brief Reports and Preliminary Communications

L. R. Morgan,* L. E. Posey, J. Rainey, J. Bickers, D. Ryan, R. Vial, and E. W. Hull[4]

Ifosfamide is an isomer of cyclophosphamide that has demonstrated antitumor activity in patients with carcinomas of the lung, gastrointestinal system, and genitourinary system (1,2).

The most serious and dose-limiting toxic effect of ifosfamide is hemorrhagic cystitis, which occurs in 40%–50% of the patients (3–8). Rodriguez et al (4) and Costanza et al (5) have recently published their data on the administration of ifosfamide in a dose-fractionated schedule in advanced malignancies and with reduced bladder toxicity.

The present study describes a well-tolerated and effective dose schedule for ifosfamide employing an intermittent 5-day loading course followed by a weekly schedule in patients with bronchogenic carcinoma. All patients received a reducing agent, ascorbic acid, in an attempt to reduce the drug's toxicity on the genitourinary system (6).

METHODS

Patients eligible for this study had histologically confirmed advanced bronchogenic carcinoma. Some patients had been previously treated and had demonstrated refractoriness to therapy. Four patients had been treated with doxorubicin and mitomycin; four had been treated with cyclophosphamide; and six had been treated with 5-FU, thiotepa, and methotrexate. To enter the study, patients could not have received chemotherapy or radiotherapy for 3 weeks prior to ifosfamide therapy. All patients had adequate renal function (serum creatinine < 2 mg/100 ml or creatinine clearance > 70 ml/minute), and most had normal liver functions (bilirubin < 2 mg/100 ml) and adequate bone marrow functions (peripheral absolute granulocyte count ≥ 2000/mm³ and platelet count ≥ 125,000/mm³). Ifosfamide was administered at a dose of 1.2 g/m² daily for 5 days and then once weekly. All patients received 250 mg of ascorbic acid orally three times a day and 500 mg at bedtime on the days of ifosfamide therapy. If toxicity was observed, the dose for that patient was reduced by 25% in subsequent courses. Ifosfamide was dissolved in 150–200 ml of 5% dextrose solution and infused over 15–30 minutes. Iv and oral hydration was administered during therapy. Bladder catheterizations were not required; the patients were urged to void frequently, every 2 hours while awake during therapy. A urinalysis was performed prior to each daily dose and on a weekly basis. If the urine sediment contained > 5 rbcs/high-power field, therapy was withheld that day. Attempts to maintain the urine pH at < 5.5 were made on each patient. In patients demonstrating fullness in the suprapubic region, dysuria, incontinence, or frequency, sulfonamide antibiotics and antispasmodic agents were used. Diuretics were not used to increase urinary output. The drug was supplied by Mead Johnson Research Center, Evansville, IN, and the National Cancer Institute, Bethesda, MD. All patients, according to institutional policies, signed an informed consent.

Tumor measurements were recorded in centimeters as the largest diameter and its perpendicular applied at the widest point of the tumor. The definition of complete response (CR) was the disappearance of all evidence of tumor. Partial response (PR) was defined as a > 50% reduction in the sum of the products of the maximum perpendicular tumor diameters, maintained for at least 4 weeks.

RESULTS

Fifty-one patients were entered in the study. Forty patients (23 males and 17 females) ranging in age from 32 to 79 years (median, 46) were evaluable for response and received at least 6 weeks of therapy. Responses are shown in table 1. Duration of CR in the patient with adenocarcinoma was 30 weeks. The

[1]Received Aug 2, 1978; revised Sept 25, 1980; accepted Oct 31, 1980.

[2]Supported in part by Mead Johnson Research Center, Evansville, IN.

[3]Presented in part at the Ifosfamide Symposium, Düsseldorf, West Germany, Mar 23, 1977.

[4]Departments of Pharmacology and Medicine, Louisiana State University Medical Center, New Orleans.

Reprint requests to: L. R. Morgan, MD, PhD, Department of Pharmacology, Louisiana State University Medical Center, 1901 Perdido St, New Orleans, LA 70112.

TABLE 1.—Results of 5-day ifosfamide loading with weekly maintenance

	No. of evaluable patients	No. of patients with—			Median survival (mos)	
		CR	PR	NR*	Responders	Nonresponders
Squamous cell carcinoma	13		4	9	9.0	2.5
Small cell carcinoma (oat cell)	9		4	5	6.5	3.5
Adenocarcinoma	16	1	4	11	15.0	4.5
Large cell carcinoma	2		1	1	7.0	4.0

*NR = no response.

PRs have lasted 15–42 weeks. One PR and one disease stabilization were seen in two of four patients receiving prior adequate cyclophosphamide therapy. The one responder (PR) was a patient with oat cell carcinoma that had relapsed after responding to cyclophosphamide for 3.5 months. Two patients responded who had failed previous doxorubicin and mitomycin treatment. Survival data are outlined in table 1.

All 51 patients in the study were evaluated for side effects. Of the 11 patients evaluated for toxicity only, six had hematuria, two had severe vomiting, and three died from progressive cancer. Hematuria occurred in 15 patients: 11 had microscopic hematuria with dysuria and four had gross hematuria. No azotemia or renal dysfunctions were observed. There were no drug-related deaths.

Five patients had mental confusion that cleared several hours after drug administration. Nausea and vomiting were noted in three patients. Thirty-six patients had alopecia. A median leukopenia of 2.5×10^3 cells/mm^3 was noted in six patients (range, 1.9–3.6×10^{-3} cells/mm^3) and a median thrombocytopenia of 83×10^3 cells/mm^3 was observed in three (range, 75–123×10^{-3} cells/mm^3). The median time for myelosuppression occurred at Day 10 (range, 6–16) and was reversed within 6–8 days.

Seven males in their sixth decade of life, who were in good physical condition and who gave a history of presently active sex lives, demonstrated increased libido. Physical examinations failed to reveal any abnormalities.

DISCUSSION

This study was undertaken to investigate the feasibility of administering ifosfamide in a weekly schedule that would reduce bladder toxicity while preserving therapeutic efficacy. It is not a controlled study but demonstrates that ascorbic acid can be used with ifosfamide with good results.

In the present study, ifosfamide produced a 35% objective response rate. This does not include 12% of the patients who demonstrated a stabilization of disease and who were included with the nonresponders. Prior treatment with radiation therapy or chemotherapy made no difference. Two patients who responded to ifosfamide had previously responded to cyclophosphamide but had then regressed.

The dose-limiting toxicity, as previously noted, is both renal and gastrointestinal (6). Severe nausea and vomiting caused dehydration, resulting in decreased urine output and bladder problems in several patients. However, $< 10\%$ of the patients experienced significant bladder problems. This is in contrast to the 60% renal toxicity reported by others (1,2). No special catheterizations were needed. Rodriguez et al (4) and Costanza et al (5) have been successful in administering ifosfamide over a 5-day period with oral hydration and repeating it every 21 days with reduced bladder toxicity.

The present study suggests that the administration of ifosfamide in daily doses of 1.2 g/m^2 for 5 days and then once weekly along with ascorbic acid is effective and tolerated in patients with bronchogenic carcinoma (6). Although there were four patients with gross hematuria, reducing agents such as ascorbic acid may be useful in reducing bladder irritation from ifosfamide and its metabolites. Less hematuria was seen than in the initial study (6). A randomized study of ifosfamide with or without reducing agents is in progress. Because of the therapeutic efficacy of ifosfamide and its mild myelosuppressive effects, particularly with sparing of the platelets, ifosfamide may have an important role in combination chemotherapy.

REFERENCES

1. BROCK N. Pharmacological studies with ifosfamide—a new oxaz-phosphorine compound. *In* Proceedings of the Seventh International Congress of Chemotherapy, Prague, 1971 (Hejzlar M, Semonsky M, and Masak S, eds). Munich, West Germany, Urban & Schwarzenberg, 1972, pp 749–756.
2. SCHEEF E. Problems, experience and results of clinical investigations with ifosfamide. *In* Proceedings of the Seventh International Congress of Chemotherapy, Prague, 1971 (Hejzlar M, Semonsky M, and Masak S, eds). Munich, West Germany, Urban & Schwarzenberg, 1972, pp 797–800.

3. Van Dyk JJ, Falkson HC, Van Der Merwe AM, et al. Unexpected toxicity in patients treated with iphosphamide. Cancer Res 32:921–924, 1972.
4. Rodriguez V, Bodey GP, Freireich EJ, et al. Reduction of ifosfamide toxicity using dose fractionation. Cancer Res 36:2945–2948, 1976.
5. Costanza JJ, Gagliano R, Loukas D, et al. Ifosfamide in recurrent or disseminated lung cancer. Cancer 41:1715–1719, 1978.
6. Morgan LR, Posey LE, Rainey J, et al. Ifosfamide in the treatment of carcinoma of the lung. Clin Res 24:512, 1976.
7. Bremner DN, McCormick JS, and Thomson JWW. Clinical trial of isophosphamide (NSC-109724)—results and side effects. Cancer Chemother Rep 58:889–891, 1974.
8. Cohen MH, Creaven PJ, Tejada F, et al. Phase I clinical trial of isophosphamide (NSC-109724). Cancer Chemother Rep 59:751–755, 1975.

Combined Radiotherapy and Bleomycin for Advanced Head and Neck Cancers: Results of a Phase I Pilot Study[1,2,3]

Brief Reports and Preliminary Communications

Ivan J. Silverberg,* Theodore L. Phillips, Karen K. Fu, and Paul Y. M. Chan[4]

SUMMARY

Forty-two patients with inoperable stage III or IV head and neck epithelial tumors were entered in a phase I pilot study to evaluate the toxicity and efficacy of combined radiotherapy and bleomycin for advanced head and neck cancers. Twenty-three patients (52%) achieved complete remissions and 18 patients (42%) had > 50% regression. Most patients tolerated bleomycin at a dose of 5 units twice a week, with a full course of radiotherapy delivered at 180 rads/day, 5 days/week. Although the complete remission rate appears to be higher than with radiotherapy alone and 13 of the 23 complete remissions (57%) lasted > 1 year, methods of prolonging the duration of remission need to be developed.

[Cancer Treat Rep 65:697–698, 1981]

Inoperable head and neck tumors are seldom controlled by radiotherapy alone. Methotrexate, 5-FU, and other agents have been tried prior to and concurrently with irradiation, with disappointing results (1–5). Bleomycin, which is active against squamous cell carcinoma when used alone, is a known radiopotentiator and is also relatively nonmyelosuppressive (6).

To evaluate the toxicity and efficacy of combined radiotherapy and bleomycin for advanced head and neck cancers, the University of California School of Medicine and the Mount Zion Hospital and Medical Center in San Francisco, together with the Kaiser Permanente Medical Center in Los Angeles (under the sponsorship of the Western Cancer Study Group), conducted the phase I pilot study reported here.

MATERIALS AND METHODS

Between January 1972 and December 1975, 42 patients with inoperable stage III or IV epithelial head and neck tumors were entered in this pilot study: 29 had had no prior treatment, seven had disease recurrent after surgery, and six had been previously irradiated.

To be eligible for the study, patients were required to have adequate renal, hepatic, hematopoietic, and pulmonary functions and a minimum Karnofsky performance status of 60%. During treatment, patients were examined at least twice weekly, with careful evaluation of pulmonary status, mucositis, and measurement of tumor size. Blood cell counts were done weekly, chest roentgenograms were obtained every 2–4 weeks, and chemistry panels were obtained monthly.

Radiotherapy was administered by ^{60}Co or 4-mev linear accelerator to the primary tumor site, adjacent tissues, and involved lymph nodes. Patients with no prior irradiation received a dose of 6500 rads delivered at 180 rad-fractions, 5 daily fractions/week. Clinically uninvolved adjacent tissue and nodal areas received a minimum dose of 5000 rads in 5–6 weeks. Previously irradiated patients were given reduced total doses at similar rates and schedules.

Bleomycin was administered on the first day of irradiation as an iv bolus. Nineteen patients received 15 units twice a week; 18 received 5 units twice a week; two received 2 units three times a week; one received 15 units once a week; and one received 10 units twice a week.

[1]Received Aug 28, 1978; revised Oct 21, 1980; accepted Nov 20, 1980.

[2]Supported in part by Public Health Service grant CA-01442 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Presented at the Meeting of the American Society of Clinical Oncologists, Toronto, Ontario, Canada, May 3–4, 1976.

[4]The Cancer Research Institute (*I. J. Silverberg*) and the Department of Radiation Oncology (*T. L. Phillips* and *K. K. Fu*), University of California, San Francisco; Mount Zion Hospital and Medical Center, San Francisco, CA (*I. J. Silverberg*); and Kaiser Permanente Medical Center, Los Angeles, CA (*P. Y. M. Chan*).

**Reprint requests to:* Ivan J. Silverberg, MD, 45 Castro St, San Francisco, CA 94114.

Chemotherapy and radiotherapy were interrupted for grade 3+ or 4+ stomatitis until toxicity cleared. Patients who developed pulmonary infiltrates received no further chemotherapy, but radiotherapy was continued whenever clinically feasible. Upon completion of the combination therapy, the individual investigators were allowed to give additional chemotherapy as they felt was clinically indicated; 14 patients received such therapy, usually at relapse. Followup times ranged from 21 to 69 months.

RESULTS

Of the 42 patients, 23 (52%) had complete responses (at least 30 days' duration) and 18 had partial responses (> 50% tumor regression).

Of the 29 previously untreated patients, 14 had complete responses, 14 had partial responses, and one had < 50% tumor regression. In complete responders radiotherapy doses ranged from 4997 to 7546 rads, with a median of 6649 rads and a mean of 6518 rads. Bleomycin doses ranged from 20 to 255 units, with a mean of 93 units. In partial responders radiotherapy doses ranged from 4620 to 7360 rads, with a median of 6480 rads and a mean of 6342 rads. Bleomycin doses ranged from 36 to 180 units, with a mean of 91 units. Of the six previously irradiated patients, four had complete responses and two had partial responses. Radiotherapy doses ranged from 3880 to 5240 rads, with a mean of 4700 rads. Of the seven patients with recurrent disease after surgery, five had complete responses and two had partial responses.

Three patients had abnormal chest roentgenograms during treatment. Only one of these had lung changes histologically compatible with bleomycin toxicity. Grades 3 and 4 mucositis occurred at a higher rate in patients treated with 15 units of bleomycin twice a week (ten of 19 patients) than in those receiving 5 units twice a week (six of 18 patients). Of the 19 patients who received 15 units of drug twice a week, 14 required a > 1-week treatment extension. Of the 18 patients receiving bleomycin at 5 units twice a week, only eight (44%) required a > 1-week treatment extension.

As of July 1, 1978, the duration of survival for the 23 complete responders ranged from 85 to 1076+ days, with a median of 392 days. Five patients were still alive and in complete remission. The range of survival for partial responders was 59–581 days, with a median of 202 days.

DISCUSSION

We found that bleomycin doses > 5 units twice a week resulted in prolonged treatment time due to severe mucositis. This enhanced mucositis is consistent with observations of other investigators (7,8). We found no significant difference in the complete response rate with respect to bleomycin dose and rate and no correlation between the degree of mucositis and response. Complete responses were of short duration. Although 13 of the 23 complete remissions lasted 1 year, at the time of re-evaluation (July 1, 1978) only five patients remained disease-free. Our observation is similar to that of Kapstad (7). It appears that some form of maintenance therapy after completion of radiotherapy is needed to consolidate and prolong complete remission. Such maintenance therapy is currently under investigation in a randomized clinical trial sponsored by the Northern California Oncology Group.

REFERENCES

1. Friedman M. The treatment of squamous cell carcinoma of the head and neck with combined methotrexate and irradiation. *In* Frontiers of Radiation Therapy and Oncology (Vaeth JM, ed). New York, NY. S. Karger, 1969, pp 106–115.
2. Kramer S. Methotrexate and radiation therapy in the treatment of advanced squamous cell carcinoma of the oral cavity, oropharynx, supraglottic larynx and hypopharynx. Can J Otolaryngol 4:213–218, 1975.
3. Lo TCM, Wiley AL, Ansfield FJ, et al. Combined radiation therapy and 5-fluorouracil for advanced squamous cell carcinoma of the oral cavity and oropharynx: a randomized study. Am J Roentgenol Radiat Ther Nucl Med 126:229–235, 1976.
4. Gollin FF, and Johnson RO. Pre-irradiation 5-FU infusion in advanced head and neck carcinomas. Cancer 24:768–770, 1970.
5. Friedman M, De Narvaes FN, and Daly JF. Treatment of squamous cell carcinoma of the head and neck with combined methotrexate and irradiation. Cancer 26:711–721, 1970.
6. Blum RH, Carter S, and Agre K. A clinical review of bleomycin—a new antineoplastic agent. Cancer 31:903–914, 1973.
7. Kapstad B. Treatment of squamous cell carcinoma of the head and neck region with cobalt and bleomycin. Int J Radiat Oncol Biol Phys 4:91–94, 1978.
8. Shanta V, and Krishnamurthi S. Combined therapy of oral cancer. Bleomycin and radiation: a clinical trial. Clin Radiol 28:427–429, 1977.

Neocarzinostatin: Report of a Phase II Clinical Trial[1,2]

Brief Reports and Preliminary Communications

Eugene M. McKelvey,* William Murphy, Axel Zander, and Gerald P. Bodey[3]

SUMMARY

Fifty-three patients received intensive treatment with neocarzinostatin in doses of 3500 units/m² by iv bolus infusion daily for 5–14 days. The response rate for 22 patients with leukemia was 9%. One complete and one partial remission were observed among nine patients with chronic myelogenous leukemia in blast cell crisis. None of the 31 patients with solid tumors responded to treatment. With this dose schedule, prolonged thrombocytopenia and cumulative bone marrow toxicity limit the intensity and duration of neocarzinostatin therapy. Acute allergic reactions occurred in 28% of the treatment courses, and three patients developed anaphylaxis during the second or third course of therapy.

[Cancer Treat Rep 65:699–701, 1981]

Neocarzinostatin is a single-chain polypeptide antibiotic which has been shown to be active in the treatment of patients with acute leukemia (1–5). Previous studies have established that doses of 2000–3500 units/m² daily for 5 days can be tolerated by patients with leukemia and by those with solid tumors (3–8). In a phase I trial using various doses of neocarzinostatin on a 5-day schedule (4), we observed four complete or partial responses among 27 patients with leukemia. Neocarzinostatin has a short plasma half-life, 72 minutes (8), but in that study, continuous infusion offered no significant advantage over 5-day intermittent administration. Accordingly, this phase II trial was undertaken using intensive intermittent neocarzinostatin therapy at maximum tolerated doses to determine the efficacy of treatment in patients with acute leukemia or chronic myelogenous leukemia in blast cell crisis and in those with selected solid tumors which are reportedly sensitive to the drug (5–8).

PATIENTS AND METHODS

Fifty-three patients were treated with 98 courses of neocarzinostatin by rapid iv infusion. Diagnoses included acute myelogenous leukemia (eight patients), chronic myelogenous leukemia in blast cell crisis (nine), and acute lymphocytic leukemia (five). The 31 patients with solid tumors in this study had the following diagnoses: renal carcinoma (19), hepatoma (five), bile duct carcinoma (four), and lymphoma (three). All leukemia patients showed progressive acute disease with > 40% blast cells, > 50% cellularity in the bone marrow, and circulating blast cells in the peripheral blood. Each patient had previously received intensive combination chemotherapy for acute leukemia.

Patients with leukemia were treated with 3500 units/m² of neocarzinostatin daily until bone marrow hypoplasia occurred or for a maximum of 14 days. These patients received a median of 7 days of therapy per course. Patients with solid tumors were initially given similar doses of neocarzinostatin in 5-day courses. Treatment was repeated as soon as the patients had recovered from myelosuppression. In patients with solid tumors, this required peripheral blood counts of at least 2000 granulocytes and 100,000 platelets/mm³. Patients with leukemia were given additional neocarzinostatin therapy when their bone marrow cellularity was at least 20% and there was evidence of increasing blast cells in either the bone marrow or the peripheral blood. The median interval between courses for all patients in this study was 33 days. At the second and subsequent courses of therapy, the intensity of neocarzinostatin treatment in patients with leukemia was adjusted by varying the duration of the treatment course. Although all patients with solid tumors continued to receive 5 days of therapy, the dose of neocarzino-

[1]Received Sept 12, 1980; revised Oct 30, 1980; accepted Nov 18, 1980.

[2]Supported by contract N01-CM-43801 from the National Cancer Institute, National Institutes of Health, Bethesda, MD.

[3]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

*_Reprint requests to:_ Eugene M. McKelvey, MD, Department of Developmental Therapeutics, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, TX 77030.

statin was adjusted to maximum tolerated levels: nine patients had a dose reduction to 2400 units/m², and the dose for two patients was increased to 6500 units/m².

The following criteria were used to evaluate therapeutic response. For patients with acute leukemia, a complete remission was defined as the presence of a normocellular bone marrow with < 5% blast cells, a normal peripheral blood count, and the disappearance of all clinical signs and symptoms of disease. Patients who had < 25% blast cells in the bone marrow and a normal peripheral blood count were considered to have a partial remission. For patients with solid tumors, a response was defined as at least a 50% decrease in the sum of the products of two perpendicular diameters for all measured lesions.

RESULTS

Only two responses to neocarzinostatin therapy were observed in this study. These two patients with chronic myelogenous leukemia achieved the maximum response (one complete remission and one partial remission) after two courses of neocarzinostatin of 7 days' duration each. Although neocarzinostatin was continued for two additional maintenance treatments, the patients promptly relapsed at 6 weeks and 5 weeks, respectively.

No additional responses were observed either among the 13 patients with acute leukemia or the 31 patients with solid tumors (table 1). These findings are not significantly different from those in our previous phase I study (4), in which 14% responses were noted among a comparable group of 22 patients with acute leukemia.

Myelosuppression was the most common toxic effect resulting from neocarzinostatin therapy. Wbc counts < 2000/mm³ and/or platelet counts < 100,000/mm³ were noted in 50% of the patients with solid tumors who were in this study. Granulocytopenia was only mild to moderate. A peripheral granulocyte count < 1000/mm³ was observed in only six of 64 treatment courses. In contrast, severe platelet suppression (< 50,000/mm³) occurred in 27% of these courses. The delay in bone marrow recovery following neocarzinostatin therapy was of even greater concern. Prolonged thrombocytopenia, defined as a platelet count < 100,000/mm³ for more than 4 weeks was noted in 17% of the patients with solid tumors following the first treatment course and in 41% after subsequent courses of therapy. These findings suggest that there is cumulative myelosuppression with the doses of neocarzinostatin used in this study.

Immediate allergic reactions (chills, sweating, and dizziness) were observed during 28% of the treatment courses. Symptoms were usually controlled by premedication with an antihistamine. However, three patients had anaphylactic reactions (cyanosis, wheezing, and hypotension) following the second or third course, which necessitated discontinuation of neocarzinostatin therapy. In the preceding phase I trial (4), it was noted that allergic reactions were more frequent with intermittent (20%) than with continuous (2%) neocarzinostatin administration.

TABLE 1.—Neocarzinostatin response rates in patients with solid tumors*

Tumor	This study	American literature	Japanese studies	Total response	(%)
Bile duct carcinoma	0/4	0/1	—	0/5	
Hepatoma	0/5	10/39	0/8	10/52	(20)
Lymphoma	0/3	2/6	—	2/9	(22)
Renal carcinoma	0/19	0/2	—	0/21	
Bladder carcinoma		1/20	28/60	29/80	(36)
Breast carcinoma		0/10	0/3	0/13	
Cervical carcinoma		—	0/12	0/12	
Colon carcinoma		0/26	0/15	0/41	
Lung carcinoma		1/22	0/10	1/32	(3)
Melanoma		1/12	1/3	2/15	(13)
Ovarian carcinoma		0/1	0/11	0/12	
Pancreatic carcinoma		0/5	11/88	11/93	(12)
Prostatic carcinoma		0/15	—	0/15	
Sarcoma		1/11	—	1/11	(9)
Stomach carcinoma		0/1	6/217	6/218	(3)
Other		0/22	3/16	3/38	(8)
Total response (%)	0/31	16/193 (8)	49/443 (11)	65/667	(10)

*Values = No. of patients who had either complete or partial response/No. of patients treated (refs 1–12).

These observations are consistent with other reports in the literature in which immediate allergic reactions occurred in 24%–43% of the patients receiving intermittent neocarzinostatin, with anaphylaxis in 3%–6% of the patients. This has usually occurred after prior exposure to the drug. These immediate allergic reactions seem particularly common with high-dose, intermittent neocarzinostatin therapy (2,5,6,9,10).

DISCUSSION

Continued interest has been shown in neocarzinostatin since it was first identified in Japan more than 15 years ago. While the Japanese have reported complete remission rates of 14%–39% in patients with acute leukemia, studies in the US have generally shown lower levels of response (2,5,6,9).

In attempting to improve the results of neocarzinostatin therapy, various dose schedules have been used. The schedule most commonly used has been intermediate doses of neocarzinostatin given by iv bolus injections daily for 5 days. Since neocarzinostatin is a low molecular weight polypeptide which is rapidly excreted in the urine, administration by continuous infusion was investigated. Although the frequency of allergic reactions was reduced and larger doses of neocarzinostatin could be given, the response rate for patients with acute leukemia was not significantly improved (2,4,6,9). The degree of myelosuppression encountered with neocarzinostatin therapy is variable over a relatively wide dose range. The present study was designed to use maximally tolerated doses of neocarzinostatin for each individual patient. While this resulted in a more intensive program for patients with acute leukemia, only two patients with solid tumors could tolerate a substantial dose increase.

This study has again confirmed that neocarzinostatin has some activity in the treatment of patients with leukemia. However, even with this more intensive neocarzinostatin therapy, the rate of response in leukemia patients was the same as that observed in our earlier phase I trial (4). Neocarzinostatin may be of value in the treatment of chronic myelogenous leukemia in blast cell crisis. The observation of two responses among our nine patients is encouraging since this disease does not generally respond to currently available chemotherapeutic agents, but the results of intensive treatment in other forms of acute leukemia are disappointing.

Neocarzinostatin, as used in this study, does not appear to be an effective treatment for patients with solid tumors. Among our patients with solid tumors, the median interval between courses of therapy was 33 days. In our experience and that of other investigators, problems with prolonged thrombocytopenia and cumulative toxicity preclude the use of neocarzinostatin in a sustained or more intensive treatment program (6,8). As shown in the accompanying table, relatively few responses have been reported to date among patients with solid tumors (1–10). Possible exceptions are the report by Falkson et al (7), using neocarzinostatin in predominantly African patients with malignant hepatoma, and the studies in patients with bladder cancer (10–12). Some of the latter patients received intravesicular therapy to further augment the high urinary drug levels which result from the rapid renal clearance of neocarzinostatin.

In our phase I trial, cumulative toxicity was not as evident at doses of 1800–2400 units/m^2 × 5 days. Thus, it may be possible, with lower intermittent doses or with other less intensive schedules, to use neocarzinostatin more frequently and to continue the treatment program for longer periods of time. These considerations may be particularly relevant to the design of combination therapy for the treatment of patients with chronic myelogenous leukemia in blast cell crisis.

REFERENCES

1. Rivera G, Howarth C, Aur RJA, et al. Phase I study of neocarzinostatin in children with cancer. Cancer Treat Rep 62:2105–2107, 1978.
2. Ohnuma T, Nogeire C, Cuttner J, et al. Phase I study with neocarzinostatin. Tolerance to two hour infusion and continuous infusion. Cancer 42:1670–1679, 1978.
3. Hiraki K, Kamimura O, Takahashi I, et al. Neocarzinostatin. A new chemotherapeutic approach to acute leukemia. Nouv Rev Fr Hematol 13:445–451, 1973.
4. McKelvey E, Burgess M, McCredie K, et al. Neocarzinostatin: a phase I clinical trial with five-day intermittent and continuous infusions. Cancer 44:1182–1188, 1979.
5. Legha S, Von Hoff D, Rozencweig M, et al. Neocarzinostatin (NSC 157365): a new chemotherapeutic compound. Oncology 33:265–270, 1976.
6. Griffin T, Comis R, Lokich J, et al. Phase I and preliminary phase II study of neocarzinostatin. Cancer Treat Rep 62:2019–2025, 1978.
7. Falkson G, Von Hoff D, Klaassen D, et al. A phase II study of neocarzinostatin (NSC 157365) in malignant hepatoma. Cancer Chemother Pharmacol 4:33–36, 1980.
8. Comis R, Griffin T, Raso V, et al. Neocarzinostatin: initial clinical and pharmacologic studies in the United States. Recent Results Cancer Res 63:261–271, 1978.
9. Issell B, Prestayko A, Comis R, et al. Zinostatin (neocarzinostatin). Cancer Treat Rev 6:239–249, 1979.
10. Natale R, Yagoda A, Watson R, et al. Phase II trial of neocarzinostatin in patients with bladder and prostatic cancer. Cancer 45:2836–2842, 1980.
11. Asahi T, Ike N, Takamoto H, et al. The treatment of bladder tumors with neocarzinostatin. Nishinippon Hinyokika 41:91–94, 1979.
12. Sakamoto S, Ogata J, Ikegami K, et al. Chemotherapy for bladder cancer with neocarzinostatin evaluation of systemic administration. Eur J Cancer 16:103–113, 1980.

Oral VP-16-213 in Transitional Cell Carcinoma of the Bladder: A Phase II Study[1]

Brief Reports and Preliminary Communications

Jytte Panduro, Mogens Hansen, and Heine Høi Hansen[2,*]

Treatment with surgery and/or radiotherapy is usually successful as regards the primary lesion in transitional cell carcinoma of the bladder, and treatment failure is related to the presence of extravesical disease in > 50% of the patients with relapse after primary local treatment. Therefore, additional modes of therapy are needed to achieve further advances in the management of patients with bladder cancer.

The most active single agents against transitional cell carcinoma are cisplatin (1), mitomycin C (2), cyclophosphamide (3), doxorubicin (4), and methotrexate (5), with a response rate of 20%–35%. Of the epipodophyllotoxin derivatives, VM-26 has also shown activity in bladder cancer. Since VP-16-213, another such derivative, can be administered orally, a phase II study of that agent in patients with bladder cancer seemed to be indicated.

MATERIALS AND METHODS

All patients included in this study were previously treated with high-voltage radiation in a dose of 6000 rads to the bladder and perivesical tissues in a 6-week period. None of the patients had previously received cytostatic drugs. A prerequisite for inclusion in the study was clinically or radiographically measurable distant metastases or cystoscopically evaluable local recurrence. Bone metastases were not considered as measurable parameters. Hematologic requirements were wbc counts > 3500/μl and platelet counts > 125,000/μl. Patients had to be < 75 years old, with serum creatinine < 200 μmols/liter, and had to give informed consent to the treatment.

VP-16-213 was administered as drinking ampules (6), each containing 100 mg of VP-16-213 (supplied by Sandoz Ltd). The initial dose was 130 mg/m² given daily for 5 days every 3 weeks, with dose modifications according to hematologic status. The dose was reduced by one fourth if the wbc counts were 2500–3500/μl or the platelet counts were 70,000–100,000/μl. If wbc counts consistently remained above these levels during two cycles, doses were increased by 25%. If wbc counts were < 2500/μl or platelet counts were < 70,000/μl, treatment was withheld and counts were done weekly. When the counts rose above these levels, VP-16-213 treatment was reinstituted with a dose reduction of one fourth. Treatment was continued until progression of the disease. Partial response was defined as at least a 50% reduction of the product of two perpendicular diameters, while progression of disease was similarly defined as a 50% increase or the emergence of new lesions. No change was recorded if neither partial response nor disease progression occurred within three treatment series.

RESULTS

Eleven patients with local recurrences and eight patients with distant metastases either to subcutaneous tissue (one patient), liver (one), or lungs (six) were included in the study. The treatment results are summarized in table 1. Three patients were not evaluable because of non-drug-related death within 3 weeks. Only one patient (6%; 95% confidence limits: 0–30%) responded. Several subcutaneous nodules, of which one was verified as malignant at biopsy, disappeared completely within the first 6 weeks. Recurrence was observed after 9 months. One patient was observed during treatment for 18 months with no change of metastases in the lungs.

Toxicity

Five patients developed leukopenia (< 2500 cells/μl) during the first course of treatment. One patient died with progressive disease and multiple abscesses in the abdominal wall and in the perivesical tissues. The terminal phase was preceded by a wbc count < 1000/μl following the third course of treatment.

[1]Received Dec 12, 1979; revised Sept 12, 1980; accepted Sept 23, 1980.

[2]Radiumstationen, Finsen Institute, Copenhagen, Denmark.

Reprint requests to: Dr. Heine H. Hansen, Department of Chemotherapy R II-V, Finsen Institute, Strandboulevarden 49, 2100 Copenhagen, Denmark.

TABLE 1.—Results of VP-16-213 treatment

Tumor type	No. of patients					
	Total	With complete response	With partial response	With no change	With disease progression	Not evaluable
Local recurrence	11	0	0	6	4	1
Distant metastases	8	1*	0	4	1	2

*Duration of the response was 9 mos.

Gastrointestinal toxicity evidenced by nausea and stomatitis occurred in most patients but in no case resulted in discontinuation of treatment. All patients had almost total alopecia, occurring after two or three treatment courses. According to the schedule, the dose of VP-16-213 was increased by 25% after two series in two patients, but it had to be reduced in five patients.

DISCUSSION

The epipodophyllotoxin derivative VM-26 was shown by the European Organization for Research on Treatment of Cancer to be active in bladder cancer (7). The tumor activity was subsequently substantiated both in patients with local bladder cancer (8) and in patients with advanced stages of the disease (9,10). In the latter study, one complete remission and one partial remission were obtained among 11 patients treated with VM-26 as a single agent. Using VP-16-213, only one of 16 evaluable patients in the present study responded. In addition, neither Falkson et al (11) nor Creaven et al (12) observed any response in four patients and one patient, respectively, with bladder cancer treated with VP-16-213. Thus, VP-16-213 does not appear to be superior to VM-26. A reliable comparison of VP-16-213 and VM-26 in bladder cancer requires a higher number of patients, most optimally in a controlled trial. At present, however, the rationale for initiating such a controlled trial is weak, particularly in patients with local disease who have previously received irradiation. In this group of patients, the response rates with both drugs as single agents are rather low, while slightly higher response rates have been observed in patients with disseminated disease (8,9).

Furthermore, in preliminary studies with combinations, VM-26 did not improve the result when combined with doxorubicin (13).

In conclusion, VP-16-213 has some activity in transitional cell carcinoma of the bladder, but the response rate is low. At present it does not appear that VP-16-213 should be considered in the treatment of bladder cancer, either as a single agent or in combinations.

REFERENCES

1. YAGODA A, WATSON RC, GONZALEZ-VITALE JC, ET AL. *Cis*-dichlorodiammineplatinum(II) in advanced bladder cancer. Cancer Treat Rep 60:917–923, 1976.
2. EARLY K, ELIAS EG, MITTELMAN A, ET AL. Mitomycin C in the treatment of metastatic transitional cell carcinoma of urinary bladder. Cancer 31:1150–1153, 1973.
3. MERRIN C, CARTAGENA R, WAJSMAN Z, ET AL. Chemotherapy of bladder carcinoma with cyclophosphamide and adriamycin. J Urol 114:884–887, 1975.
4. YAGODA A, WATSON RC, WHITMORE WF, ET AL. Adriamycin in advanced urinary tract cancer. Cancer 39:279–285, 1977.
5. HALL RR, BLOOM HJG, FREEMAN JE, ET AL. Methotrexate treatment for advanced bladder cancer. Br J Urol 46:431–438, 1974.
6. LAU ME, HANSEN HH, NISSEN NI, ET AL. Phase I trial of a new form of an oral administration of VP-16-213. Cancer Treat Rep 63:485–487, 1979.
7. EUROPEAN ORGANIZATION FOR RESEARCH ON TREATMENT OF CANCER, COOPERATIVE GROUP FOR LEUKEMIAS AND HEMATOSARCOMAS. Clinical screening of epipodophyllotoxin VM 26 in malignant lymphomas and solid tumors. Br Med J 2:744–748, 1972.
8. MECHL Z, ROVNÝ F, and SOPKOVÁ B. VM 26 (4-demethyl-epipodophyllotoxin-β-D-thenylidene glucoside) in the treatment of urinary bladder tumors. Neoplasma 4:411–414, 1977.
9. PAVONE-MACALUSO M, CARAMIA G, RIZZO FP, ET AL. Preliminary evaluation of VM-26. Eur Urol 1:53–56, 1975.
10. PAVONE-MACALUSO M, and EUROPEAN ORGANIZATION FOR RESEARCH ON TREATMENT OF CANCER GENITO-URINARY TRACT COOPERATIVE GROUP A. Single-drug chemotherapy of bladder cancer with adriamycin, VM-26 or bleomycin. Eur Urol 2:138–141, 1976.
11. FALKSON G, VAN DYK JJ, VAN EDEN EB, ET AL. A clinical trial of the oral form of 4′-demethyl-epipodophyllotoxin-β-D-ethylidene glucoside (NSC 141540), VP-16-213. Cancer 35:1141–1144, 1975.
12. CREAVEN PJ, NEWMAN J, SELAWRY OS, ET AL. Phase I clinical trial of weekly administration of 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside)(NSC-141540; VP-16-213). Cancer Chemother Rep 58:901–907, 1974.
13. RODRIGUEZ LH, JOHNSON D, HOLOYE PY, ET AL. Combination VM-26 and adriamycin for metastatic transitional cell carcinoma. Cancer Treat Rep 61:87–88, 1977.

Phase II Evaluation of Chlorozotocin in Patients With Malignant Melanoma[1,2,3]

Brief Reports and Preliminary Communications

Alan N. Houghton,* Fernando J. Camacho, Richard J. Gralla, and Robert Wittes[4,5]

Chlorozotocin is a water-soluble chloroethyl nitrosourea which has shown activity in preclinical studies in murine systems, including P388 leukemia, L1210 leukemia, and B16 melanoma (1). In phase I trials a single dose of 120 mg/m² or a divided total dose of 150–200 mg/m² produced acceptable myelosuppression and infrequent nausea and vomiting (2–5). Because nitrosoureas have activity in the treatment of melanoma, a phase II study was undertaken with chlorozotocin, an agent with greater alkylating activity than the available lipid-soluble nitrosoureas (2).

MATERIALS AND METHODS

Twenty-two patients with histologically confirmed malignant melanoma were entered in this study between September 1977 and September 1979. All patients had objectively measurable disease, platelet count > 150,000/mm³, wbc count > 4000/mm³, and Karnofsky performance status (PS) ≥ 40. Chlorozotocin was given in equally divided doses by rapid iv infusion for 5 consecutive days. The initial total dose was 200 mg/m² for patients who had not received prior chemotherapy and who had a PS ≥ 70. Patients who had been previously treated with chemotherapy and had a PS ≥ 70 were treated initially with a dose of 175 mg/m², while those with a PS < 70 received 150 mg/m². Treatments were repeated every 6 weeks if the platelet count returned to 100,000/mm³ and the wbc count returned to 3500/mm³. Therapeutic responses were defined as: *partial remission*—a ≥ 50% reduction of all measurable lesions for at least 1 month; and *minor response*—a > 25% reduction of all measurable lesions, but less than that required for a partial response. Patients were considered evaluable 6 weeks after initiation of therapy.

RESULTS

Nineteen patients were evaluated for therapeutic response, including 13 men and six women. The median age was 52 years (range, 31–82) and the median Karnofsky PS was 70 (range, 40–90). Fifteen of 19 patients had received prior chemotherapy. Fifteen patients had been previously treated with DTIC and three of these patients had also received nitrosoureas. Visceral disease was present in 17 of 19 patients (metastases to the lung in 15 patients, to the liver in five, and to the central nervous system in three). Sites of measurable disease included soft tissue in 17 patients, lung in 18, and liver in three. The number of treatment cycles received by patients ranged from one to three, with a median of one cycle per patient. Three patients were not evaluated for response; two of these patients died within 4 weeks after beginning chlorozotocin therapy and one patient was lost to followup.

Only one minor response (tumor reduction < 50%) was noted in 19 evaluable patients. The remaining 18 patients had disease progression. The minor response was observed in a 49-year-old male who had previously received adjuvant DTIC chemotherapy; a > 25% reduction was seen in the size of subcutaneous, lymph node and liver metastases; the response lasted 3 months.

The major dose-limiting toxic effect was myelosuppression, particularly thrombocytopenia, with a median platelet count nadir of 114,000/mm³ occurring on Day 28 (table 1). Wbc counts fell to < 3500/mm³ in only two patients, with a median wbc count nadir of 6700/mm³ occurring on Day 28. Myelo-

[1]Received Apr 23, 1980; revised Oct 2, 1980; accepted Oct 9, 1980.

[2]Supported in part by contract N01-CM-57043 (Division of Cancer Treatment) and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. A. N. Houghton and R. J. Gralla are American Cancer Society Junior Faculty Clinical Fellows.

[3]Chlorozotocin was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4]Developmental Chemotherapy (*R. J. Gralla*) and Solid Tumor (*A. N. Houghton, F. J. Camacho,* and *R. Wittes*) Services, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.

[5]We thank Kathleen Trainor for preparation of the manuscript.

**Reprint requests to*: Dr. Alan N. Houghton, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Hematologic toxicity in 19 patients treated with chlorozotocin

Hematologic toxicity	No. of patients
Platelet count nadir (cells/mm³)	
< 50,000	5
50,000–99,000	3
100,000–150,000	4
> 150,000	7
Wbc count nadir (cells/mm³)	
< 1000	1
1000–3500	1
> 3500	17

suppression did not result in morbidity or mortality. The agent was well-tolerated, with only four patients having mild nausea and vomiting. No renal or hepatic toxicity occurred.

DISCUSSION

Only one minor response occurred in 19 patients with melanoma treated with chlorozotocin. Most of the patients in this study had received prior chemotherapy and had visceral metastases. However, this group of patients had a good PS (median, 70) and most were able to receive therapy on an outpatient basis with few immediate side effects. Thrombocytopenia was the most frequently observed toxic manifestation (table 1).

Hoth et al (6) observed that chlorozotocin, used at a dose of 120 mg/m², produced therapeutic responses in five of 34 (14.1%) patients with malignant melanoma, but four of the five patients who responded had not received prior chemotherapy. In the present study, with no major responses observed with the same dose, chlorozotocin has a predicted true major response rate of $\leq 15\%$ ($P = 0.05$) in patients with visceral metastatic melanoma who have previously received chemotherapy. We conclude that chlorozotocin at this dose and schedule is not effective as second-line chemotherapy in the treatment of advanced malignant melanoma.

REFERENCES

1. WASSERMAN TH, SLAVIK M, and CARTER SK. Clinical comparison of the nitrosoureas. Cancer 36:1258–1268, 1975.
2. WHEELER GP, BOWDON BJ, GRIMSLEY JA, ET AL. Interrelationships of some chemical, physiochemical, and biological activities of several 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 34:194–200, 1974.
3. GRALLA. RJ, TAN CTC, and YOUNG CW. Phase I trial of chlorozotocin. Cancer Treat Rep 63:17–20, 1979.
4. HOTH D, WOOLLEY P, GREEN D, ET AL. Phase I studies on chlorozotocin. Clin Pharmacol Ther 23:712–722, 1978.
5. KOVACH JS, MOERTEL CG, SCHUTT AJ, ET AL. A phase I study of chlorozotocin (NSC-178248). Cancer 43:2189–2196, 1979.
6. HOTH D, ROBICHAUD K, WOOLLEY PV, ET AL. Phase II trial of chlorozotocin in metastatic malignant melanoma. Proc Am Assoc Cancer Res and ASCO 20:413, 1979.

Phase I Study With Cell Wall Skeleton of *Nocardia rubra* [1,2]

Brief Reports and Preliminary Communications

Yuichi Yamamura,[3] Takeshi Ogura,[3,*] Fumio Hirao,[3] Kosei Yasumoto,[4] Kenji Sawamura,[5] Shoji Hattori,[6] Yoshihiro Hayata,[7] Susumu Kishimoto,[8] Kazumasa Yamada,[9] Hisanobu Niitani,[10] and Toru Masaoka[6]

SUMMARY

Toxicity of the cell wall skeleton of *Nocardia rubra* (N-CWS) was evaluated in 60 patients with lung cancer, leukemia, and malignant melanoma who were given the agent intratumorally, intrapleurally, and intradermally in doses of 100–2000 μg. Fever was the most common toxic effect and was more frequent after intratumoral and intrapleural doses than after intradermal doses. The other predominant reaction was skin lesions such as erythema, induration, and abscess at the injection sites.

[Cancer Treat Rep 65:707–709, 1981]

The accepted clinical benefits of living BCG in cancer immunotherapy are attended by serious side effects and difficulty in dose control (1). Efforts have therefore been made to isolate its active component in order to facilitate clinical application (2,3). The present paper describes the results of our study of *Nocardia rubra* cell wall skeleton (N-CWS), which was shown in our previous studies to provide a potent immunotherapy for malignant diseases (4–7).

[1]Received May 21, 1980; revised Sept 15, 1980; accepted Oct 22, 1980.

[2]Supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture, Japan.

[3]Third Department of Internal Medicine, Osaka University Medical School, Japan.

[4]Department of Chest Surgery, Kyushu Cancer Center, Japan.

[5]Department of Surgery, Kinki Central Hospital for Chest Diseases, Japan.

[6]Center for Adult Diseases, Osaka, Japan.

[7]Department of Surgery, Tokyo Medical College, Japan.

[8]Department of Internal Medicine, Kumamoto University Medical School, Japan.

[9]Department of Internal Medicine, Nagoya University Medical School, Japan.

[10]National Cancer Center, Tokyo, Japan.

**Reprint requests to:* Dr. Takeshi Ogura, Third Department of Internal Medicine, Osaka University Hospital, Fukushima-Ku, Osaka 553, Japan.

PATIENTS AND METHODS

Immunoadjuvant.—The currently used N-CWS preparation consists basically of nocardomycolic acid, arabinogalactan, and mucopeptide. It is prepared by lyophilization and must be reconstituted with physiologic saline before use (7).

Patients and Treatment Programs.—Sixty patients were studied: 45 with lung cancer (including two with metastasis), 14 with acute leukemia (seven with myelogenous, one with promyelocytic, one with monocytic, and five with lymphoblastic), and one with malignant melanoma. They were treated at eight Japanese medical institutions. Informed consent was obtained before the study.

The present study used experimental designs of previous BCG-CWS studies as a guide (8–11). Prior to the study, all patients were tested for hypersensitivity by intradermal injection of 10 μg of N-CWS into the forearm. Of the 25 patients with inoperable lung cancer who had received chemotherapy and/or radiation therapy, 12 were given N-CWS intrapleurally, three intratumorally, and ten intradermally (table 1). Concurrent chemotherapy was given to 12 patients. Of the 20 patients who underwent surgical resection, four received preoperative N-CWS intratumorally either through a bronchofiberscope or by a percutaneous needle. One of these four patients (table 1, dagger) also received postoperative intrapleural injections. All leukemia patients who had successfully achieved complete remission with previous chemotherapy and one melanoma patient who also received chemotherapy were given intradermal N-CWS injections. In 50 patients, weekly or biweekly injections by any route were followed by monthly intradermal injections. Systemic effects of N-CWS were examined by laboratory tests including routine hematologic and serum biochemical studies performed before, during, and after the treatment. The immediate effects of injections of N-CWS on body temperature and sub-

TABLE 1.—Dose and route of N-CWS treatment*

	Lung cancer					Leukemia	Melanoma
	it		ip		id	id	id
	NS	S	NS	S	NS	NS	NS
Total	3	4†	12	17†	10	14	1
Maximum single dose (μg)							
100						3	
200	2		0	2	8	8	
300	1		8	14	2	2	1
400			2				
500			1				
800			1			1	
2000		4		1†			
Total dose (μg)							
~ 500	1		1	1	3	5	
~1000	0		2	5	5	5	
1100							1
~1500	2		2	1	2	3	
~2000		2	0	1			
~2500			7	8			
2800						1	
4000		1		1			
6000		1†					

*Values = No. of patients; it = intratumoral, ip = intrapleural, id = intradermal injection of N-CWS; NS = no surgery, S = surgery.
†One patient received both it and ip injections.

TABLE 2.—No. of adverse reactions

Reaction*	No. of reactions/ No. of injections (%)
Fever (37.0°C–39.9°C)	
Intradermal injection	8/53 (15.1)
Intrapleural injection	24/28 (85.7)
Intratumoral injection	5/7 (71.4)
Skin lesion at the injection site	
Induration	11/53 (20.8)
Erythema	10/53 (18.9)
Abscess without ulceration	5/53 (9.4)
Abscess with ulceration	2/53 (3.8)

*Other adverse reactions: lymphadenopathy, 2 patients; chest pain, 2; chills, 2.

jective complaints were checked frequently before and after the course of injection.

RESULTS

The period of study for each patient ranged from 3 to 11 weeks and the total N-CWS dose ranged from 200 to 6000 μg. The most common subjective symptom was fever of 37°C or more, which was observed in 34 (57%) patients. The duration of the fever averaged 2.1 days and varied with the route of administration (table 2). The other predominant toxic effect was skin lesions at the injection sites. Chills, chest pain, and lymphadenopathy were each observed in two patients. Laboratory tests showed transient elevation of SGOT, SGPT, and/or alkaline phosphatase in six patients (four with lung cancer and two with leukemia) during the treatment with N-CWS. The elevation of those serum enzymes was attributable to the concurrent use of anticancer drugs and anesthesia or to hepatic metastasis.

DISCUSSION

In this study the single doses of N-CWS ranged from 100 to 2000 μg. They were set without making allowance for dose/m^2 and were far lower than in animal studies in that respect, as is seen in another study for BCG-CWS (2). The most common adverse reaction at these dose levels was fever, which was persistent after intratumoral or intrapleural injections. The next most common effect was skin lesions at the injection sites. The reactions seemed to vary in severity with single N-CWS dose (intradermal, 100 to 300 μg; intratumoral and intrapleural, 200 to 2000 μg) and administration route and to subside more easily than those resulting from BCG-CWS

(8–11). None of these reactions were severe enough to necessitate discontinuation of the treatment. Although few instances of serum enzyme elevation by N-CWS have been recorded in this study, we recommend that attention be given to the possible hepatotoxicity due to the combined use of N-CWS and anticancer drugs.

REFERENCES

1. Schwarzenberg L, Simmler MD, and Pico JL. Human toxicology of BCG applied in cancer immunotherapy. Cancer Immunol Immunother 1:69–76, 1976.
2. Richman SP, Gutterman JU, Hersh EM, et al. Phase I-II study of intratumoral immunotherapy with BCG cell wall skeleton plus P_3. Cancer Immunol Immunother 5:41–44, 1978.
3. O'Connell MJ, Moertel CG, Ritts RE, Jr, et al. A comparative clinical and immunological assessment of methanol extraction residue of bacillus Calmette-Guérin versus placebo in patients with advanced cancer. Cancer Res 39:3720–3724, 1979.
4. Azuma I, Taniyama T, Yamawaki M, et al. Adjuvant and antitumor activities of Nocardia cell wall skeletons. Gan 67:733–736, 1976.
5. Ogura T, Namba M, Hirao F, et al. Association of macrophage activation with antitumor effect on syngeneic fibrosarcoma by Nocardia rubra cell-wall skeleton. Cancer Res 39:4706–4712, 1979.
6. Azuma I, Yamawaki M, Yasumoto K, et al. Antitumor activity of Nocardia cell wall skeleton preparations in transplantable tumors in syngeneic mice and patients with malignant pleurisy. Cancer Immunol Immunother 4:95–100, 1978.
7. Yamawaki M, Azuma I, Saiki I, et al. Antitumor activity of squalene-treated cell-wall skeleton of Nocardia rubra in mice. Gan 69:619–626, 1978.
8. Kishimoto S, Araki K, and Saito T. Immunotherapy with BCG or its derivatives in acute myelogenous leukemia. Gan Monogr Cancer Res 21:189–198, 1978.
9. Yamada K, Kawashima K, Morishima Y, et al. Chemoimmunotherapy of acute myelogenous leukemia in adults with BCG cell wall skeleton. Gan Monogr Cancer Res 21:199–209, 1978.
10. Yamamura Y, Sakatani M, Ogura T, et al. Adjuvant immunotherapy of lung cancer with BCG cell wall skeleton (BCG-CWS). Cancer 43:1314–1319, 1979.
11. Yasumoto K, Manabe H, Ueno M, et al. Non-specific adjuvant immunotherapy of lung cancer with cell wall skeleton of Mycobacterium bovis bacillus Calmette-Guérin. Cancer Res 39:3262–3267, 1979.

Phase II Study of Vinblastine-CCNU, Triazinate, and Dactinomycin in Advanced Renal Cell Cancer[1,2]

Brief Reports and Preliminary Communications

Richard G. Hahn,* Colin B. Begg, and Thomas Davis[2]

SUMMARY

A total of 133 patients with advanced or metastatic adenocarcinoma of the kidney were stratified according to performance status and time from initial diagnosis to recurrence and were then randomized to one of three different drug treatment arms or the crossover secondary treatment. The study drugs (combination vinblastine-CCNU and single-agent triazinate or dactinomycin) failed to provide any meaningful antitumor activity for these patients with advanced renal cell cancer.

[Cancer Treat Rep 65:711–713, 1981]

Adenocarcinoma of the kidney is the third most commonly occurring urologic cancer reported in the United States. Patients treated with surgery to control this disease have not been significantly helped, and most die from their renal cell cancer. A number of clinical trials with hormonal and cytotoxic chemotherapy have been reported (1–6). Unfortunately, no effective therapy for recurrent or advanced disease has thus far been identified in these trials. Because of the dismal prognosis and lack of effective therapy, this group of patients lends itself to phase II chemotherapy trials using new or previously untested antitumor agents. In its continuing effort to identify antitumor activity in this tumor system, the Eastern Cooperative Oncology Group (ECOG) employed two single agents: triazinate (TZT) (Baker's antifol) and dactinomycin (Act D). Because combination therapy with vinblastine and CCNU (VLB-CCNU) was noted to have definite activity in a preliminary report of a phase II trial (7), a larger confirmatory trial of this combination seemed indicated and was incorporated as the third arm of the phase II prospective trial reported here.

PATIENTS AND METHODS

The patients studied in this phase II trial were required to fulfill the following pretreatment criteria: pathologic confirmation of adenocarcinoma of the kidney; measurable advanced, inoperable local or metastatic disease; verified disease progression; ECOG performance status of ≤ 3; no chemotherapy or radiotherapy within the previous month; no prior treatment with the study drugs; adequate renal function (creatinine < 1.6 mg/100 ml); adequate

[1]Received July 1, 1980; revised Oct 27, 1980; accepted Nov 20, 1980.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, Chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Mayo Clinic, Rochester MN (*R. G. Hahn*, CA-13650); Sidney Farber Cancer Institute, Boston, MA (*C. B. Begg*, CA-23318); and Wisconsin Clinical Cancer Center, Madison (*T. Davis*, CA-21076). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia, PA (CA-18281); Baystate Medical Center, Springfield, MA (CA-20182); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia, PA (CA-13611); Abraham Lincoln School of Medicine, Chicago, IL; Jefferson Medical College, Philadelphia, PA (CA-14215); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); Medical College of Ohio, Toledo; University of Michigan, Ann Arbor (CA-21504); University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Pennsylvania Hospital, Philadelphia (CA-13613); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); University of Montreal Affiliated Hospitals, Quebec, Canada (CA-21653); University of Rochester Cancer Center, NY (CA-11083); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21692); Swiss Group, Bern, Switzerland; State University of New York—Stony Brook, (Veterans Administration Hospital), Northport, (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond, VA (CA-10572); and Yale School of Medicine, New Haven, CT (CA-15485).

*Reprint requests to: R. G. Hahn, MD, Medical Oncology and Internal Medicine, Mayo Clinic, Rochester, MN 55901.

marrow reserve (wbc count ≥ 4000/mm³ and platelet count ≥ 100,000); informed consent; no major surgery within the previous 3 weeks; adequate daily nutrition; and no serious infections.

Patient stratification prior to treatment randomization was performed according to performance status (ECOG 0 or 1 and ECOG 2 or 3) and time from initial diagnosis to detection of metastatic or recurrent disease (< 1, 1–5, and > 5 years).

Patients primarily randomized to the VLB-CCNU arm received VLB in a dose of 4.0 mg/m² iv on Days 1 and 8 of the treatment cycle and CCNU in a dose of 120 mg/m² orally on Day 1. This treatment regimen was scheduled to be repeated every 6 weeks. Patients randomized to the TZT arm received a dose of 250 mg/m² iv daily for 3 consecutive days, with the course to be repeated every 3–4 weeks. Patients randomized to the Act D arm received 0.5 mg/m² of the drug iv daily for 4 consecutive days every 4–5 weeks. In each instance, two courses of therapy were to be given unless disease progression or serious toxicity prevented such re-treatment. Hematologic and other toxicity directed a scheduled dose modification for the second and subsequent courses of therapy. After failure of the initial randomized treatment, secondary or crossover treatment was allocated on the basis of performance status and initial treatment stratification. Registration and all treatment allocations were achieved by telephone contact with a central operations office.

Patients with objective response or disease stability in accordance with previously outlined ECOG criteria for response continued receiving the initial therapy until disease progression. Patients whose disease progressed during treatment were eligible for crossover therapy if initial eligibility criteria were met and the patients desired further treatment.

Of 143 patients randomized to the initial treatment program, 51 were subsequently entered on crossover therapy and ten were excluded. Four of the 51 crossover patients were excluded from the crossover study treatment program. These 14 patients were excluded from evaluation because of failure to meet the protocol on-study eligibility criteria.

Sixty-nine percent of all patients studied had a performance score of 0 or 1, and this percent was similar in all three arms of the treatment program. The interval from initial diagnosis to evidence of recurrent or metastatic disease was < 1 year in 70% of the patients (this same figure was approximated in all the treatment arms of the study) and > 5 years in 9%. Males outnumbered females by three to one, 64% of the patients were < 60 years of age, and 68% of the group had had previous radiotherapy for some aspect of their recurrent disease. Eighty-one percent had had no prior chemotherapy, and only 12% had not had a previous definitive surgical procedure.

RESULTS

Eight responses were observed in the overall patient population. Three partial responses occurred with VLB-CCNU: two after primary therapy and one after crossover therapy (duration 8, 17, and 21 weeks, respectively). One complete response of 21 weeks occurred after primary treatment with VLB-CCNU. One complete (8½ months continuing) and two partial (17 months and 6 weeks continuing) responses occurred with TZT. One patient receiving Act D had a partial response of 9 weeks. All responders had a performance status of 0–1, and the time from initial diagnosis was < 5 years (< 1 year in five of the patients).

Survival.—Survival was measured from the date of randomization to the date of death. The overall median survival was 27 weeks. No apparent survival difference was observed between treatments, with performance status again clearly an important prognostic factor. Although the time from initial diagnosis has no statistically significant effect, there is an indication that fast-growing tumors, as one might expect, are associated with a shorter survival time.

Toxicity.—All three treatment arms produced a variety of toxic reactions, with nausea and vomiting and hematologic toxicity being the most prevalent (table 1). Initial therapy with both TZT and Act D produced a moderate degree of skin toxicity. There were four life-threatening hematologic reactions, two of these being severe marrow depression following VLB-CCNU. One TZT patient in the crossover treatment group had life-threatening marrow suppression with bleeding and a febrile response. One additional crossover patient had life-threatening myelosuppression with bleeding following Act D treatment. Two additional patients had life-threatening toxic reactions related to TZT: one had a neurologic reaction and one had a febrile reaction. No drug-related deaths occurred in either the primary or crossover treatment group.

DISCUSSION

The earlier reported response rate of 24% for combination VLB-CCNU was not observed in this study, and a low-order 6% (four of 60 patients) response rate was recorded. Previous ECOG studies of single-agent VLB and methyl-CCNU also reported a low activity. TZT and Act D, agents not previously studied formally, were also found to be

TABLE 1.—Toxic reactions

Treatment	No. of patients given agents as primary treatment	Total No. of patients treated with agents	Degree of toxicity	No. of patients with toxic effects		
				Hematologic*	Gastrointestinal	Mucocutaneous
VLB-CCNU	45	60	Moderate	13	25	4
			Severe	16	6	0
			Life-threatening	2	0	0
TZT	42	59	Moderate	2	19	10
			Severe	11	4	7
			Life-threatening	1	0	0
Act D	46	61	Moderate	8	19	8
			Severe	6	8	13
			Life-threatening	1	0	0

*Moderate = wbc count nadir of 2000–3000 cells/mm^3, platelet count nadir of 50,000–90,000 cells/mm^3; severe = wbc count nadir of 1000–2000 cells/mm^3, platelet nadir of 25,000–50,000 cells/mm^3; life-threatening = wbc count nadir < 1000 cells/mm^3, platelet count nadir < 25,000 cells/mm^3.

ineffective for renal cell cancer (three responses in 59 patients and one response in 61 patients, respectively). This study, like previously reported trials with other agents, affirms the aggressive and drug-resistant nature of renal cell cancer. Continued phase II trials in patients with this resistant and poor-prognosis cancer are obviously necessary.

REFERENCES

1. TALLEY RW. Chemotherapy of adenocarcinoma of the kidney. Cancer 32:1062–1065, 1973.
2. BLOOM HJG. Hormone-induced and spontaneous regression of renal cancer. Cancer 32:1066–1071, 1973.
3. WAGLE DJ, and MURPHY GP. Hormonal therapy in advanced renal cell carcinoma. Cancer 28:318–321, 1971.
4. WASSERMAN TH, COMIS RL, GOLDSMITH M, ET AL. Tabular analysis of the clinical chemotherapy of solid tumors. Cancer Chemother Rep Part 3, vol 6(2):399–419, 1975.
5. HAHN RG, TEMKIN NR, SAVLOV ED, ET AL. Phase II study of vinblastine, methyl-CCNU, and medroxyprogesterone in advanced renal cell cancer. Cancer Treat Rep 62:1093–1095, 1978.
6. HAHN RG, BAUER M, WOLTER J, ET AL. Phase II study of single-agent therapy with megestrol acetate, VP-16-213, cyclophosphamide, and dianhydrogalactitol in advanced renal cell cancer. Cancer Treat Rep 63:513–515, 1979.
7. DAVIS TE, and MANALO FB. Combination chemotherapy of advanced renal cell cancer with CCNU and vinblastine. Proc Am Assoc Cancer Res and ASCO 19:C-39, 1978.

Evaluation of VP-16-213, Cyclophosphamide, Doxorubicin, and Cisplatin (V-CAP) in Advanced Large Cell Lung Cancer[1,2]

Brief Reports and Preliminary Communications

Robert T. Eagan,* Stephen Frytak, William C. Nichols, James N. Ingle, Edward T. Creagan, Larry K. Kvols, and Douglas T. Coles[3]

In 1977, we reported on the results of our use of the combination of cyclophosphamide, doxorubicin, and cisplatin (CAP-I) in advanced non-small cell lung cancer (1). Sixteen patients with large cell carcinoma (LGC) of the lung were treated, with an observed regression rate of 38% (six regressions among 16 patients). Subsequent to that study, we conducted another trial using the same three-drug combination in patients with LGC of the lung (2) in which we increased the dose of cisplatin (from 40 to 60 mg/m²) and changed the method of administration (from a rapid iv infusion without mannitol to a 6-hour infusion with mannitol) (CAP-II). Of the 20 patients treated, ten (50%) had tumor regressions using the new regimen.

While the above combination regimens were being studied, single agents were also being evaluated. We found the epipodophyllotoxin derivative VP-16-213 to have activity in advanced lung cancer (3,4). In our study of the drug, only one patient with LGC of the lung was treated; this patient had a tumor regression.

We then attempted to add VP-16-213 to the CAP-I regimen (5). A trial of VP-16-213, cyclophosphamide, doxorubicin, and cisplatin (V-CAP) was conducted in patients with advanced adenocarcinoma of the lung. In that trial, VP-16-213 was administered in an unusual single-day intermittent schedule and at the somewhat low dose of 125 mg/m² (the more routine schedule being 50 mg/m²/day for 5 days). The regression rate observed was only 35% (seven regressions among 20 patients), with no improvement over the regression rate of 43% (six regressions among 14 patients) initially reported for CAP-I in patients with no prior chemotherapy. Since a more common dose schedule for VP-16-213 is 60 mg/m²/day for 5 days (total dose, 300 mg/m²), we revised the V-CAP schedule by (*a*) dividing the 60-mg/m² dose of cisplatin and giving it over 3 days at 20 mg/m² as a 1-hour infusion in 500 ml of 5% dextrose in ½ normal saline without mannitol, and (*b*) increasing the dose and days of administration of VP-16-213 to 50 mg/m²/day for 3 days. This report documents the result of that study.

MATERIALS AND METHODS

Twenty-eight patients with biopsy-proven LGC of the lung spread beyond one hemithorax and the ipsilateral supraclavicular fossa and no prior chemotherapy were entered in the study; all 28 were evaluable. The pretreatment patient characteristics are shown in table 1. The median age was 58 years, approximately two thirds had an Eastern Cooperative Oncology Group performance score (ECOG PS) of 0–1, two thirds were males, and about 50% had clearly measurable tumors. Patients were ineligible for the study for any of the following reasons: leukopenia (wbcs < 4000/mm³), thrombocy-

TABLE 1.—Patient pretreatment characteristics and regression rates

Characteristics	No. of patients	No. of regressions (%)
Entered	28	
Evaluable	28	13 (46)
Age (yrs)		
Median: 58		
Range: 32–67		
ECOG PS		
0	3	10 (53)
1	16	
2	7	3 (33)
3	2	
Males	18	10 (56)
Females	10	3 (30)
Measurable disease	13	6 (46)
Evaluable disease	15	7 (47)

[1]Received July 29, 1980; revised Oct 27, 1980; accepted Nov 13, 1980.

[2]Supported in part by contract N01-CM-57044 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Division of Medical Oncology, Mayo Clinic, Rochester, MN.

**Reprint requests to:* Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55901.

topenia (platelets < 130,000/mm³), ECOG PS of 4, serum creatinine ≥ 2.0 mg/dl, prior chemotherapy, or significant active cardiac disease.

Measurable disease was defined as a tumor with clearly defined borders not impinging on normal body structures. A complete regression (CR) was defined as the disappearance of all tumor. A partial regression (PR) was defined as a ≥ 50% decrease in the product of the longest perpendicular diameters of the lesion (or a ≥ 30% decrease in the sum of the distances that the liver extended below the costal margins in the midclavicular lines and the xyphoid). Stable disease was defined as any decrease less than that required for a CR or PR without the appearance of any new lesions. Disease progression was defined as a ≥ 25% increase in the product of the longest perpendicular diameters of the lesion (or a ≥ 15% increase in the sum of the distances that the liver extended below the costal margins in the midclavicular lines and the xyphoid) compared to the smallest previous measurement.

Evaluable disease was defined as a clearly visible and assessable tumor, but one that was not measurable mainly because of impingement on normal body structures or because of unclear borders. For evaluable disease, a CR was defined as the disappearance of all tumor, a regression was defined as a definite decrease in tumor size, stable disease was defined as no appreciable increase or decrease in tumor size and no new lesions, and disease progression was defined as any definite increase in tumor size compared to the smallest previous size. All assessments of evaluable disease required the agreement of two medical oncologists. These criteria, and their rationale, have been reported in detail elsewhere (5). A CR or PR of measurable disease is considered to be the equivalent of a CR or regression of evaluable disease.

Cisplatin was given as a 1-hour infusion in 500 ml of 5% dextrose in ½ normal saline on Days 1–3 at a dose of 20 mg/m²/day (total dose, 60 mg/m²); VP-16-213 was given as a 1-hour infusion in 500 ml of ½ normal saline on Days 1–3 (before the cisplatin) at a dose of 50 mg/m²/day (total dose, 150 mg/m²); doxorubicin was given by rapid iv injection on Day 2 at a dose of 40 mg/m²; and cyclophosphamide was given by rapid iv injection on Day 3 at a dose of 400 mg/m². Re-treatment intervals were 4 weeks. The maximum allowable total cumulative dose of doxorubicin was 450 mg/m².

During the first two courses of chemotherapy, four patients with CNS metastases received whole-brain irradiation in conjunction with their chemotherapy. The brain metastases were not used as indicator lesions.

RESULTS

Tumor regression.—Thirteen (46%) of the 28 patients had tumor regressions. There was a 47% regression rate (seven regressions among 15 patients) among the patients with evaluable disease and a 46% regression rate (six regressions among 13 patients) among the patients with measurable disease. The regression rate was higher among patients with an ECOG PS of 0–1 (53%; ten regressions among nineteen patients) than among patients with an ECOG PS of 2–3 (33%; three regressions among nine patients) and was higher among males (56%; ten regressions among 18 patients) than among females (30%; three regressions among ten patients). The primary indicator of disease in 27 of the 28 patients was the lung primary. Incidences of regression by sites of disease were as follows: primary tumor, 13 of 27; lymph nodes, five of ten; opposite lung metastases, five of seven; liver, four of six; bone, one of five; pleura, two of three; subcutaneous metastases, one of two; and adrenal glands, none of one.

Time to disease progression.—Time to disease progression was calculated from Day 1 of treatment and the median was obtained from Kaplan-Meier plots. Median time to disease progression for all patients was 142 days (range, 7–631+) and ranged from 56 days (range, 7–417+) for patients without regression to 204 days (range, 67–631+) for patients with regression.

Survival.—Survival was calculated from Day 1 of treatment and the median was obtained from Kaplan-Meier plots. Median survival for all patients was 211 days (range, 7–631+). The median survival for the regressors (268 days; range, 88–631+) was more than twice as long as that for the nonregressors (115 days; range, 7–417+).

Toxicity

Nausea and vomiting (primarily from the cisplatin) was the major unpleasant subjective toxic effect. It was moderate to severe in almost all patients. Because of the nausea and vomiting, three patients refused further treatment after one, three, and five courses, respectively. Alopecia occurred in all patients. No cardiac toxicity or drug deaths were observed. Two patients were hospitalized with leukopenia but no documented infections were found.

Myelosuppression was the most frequent and significant objective toxic effect. Following the first course of V-CAP, leukopenia was observed in 88% of patients (median wbc count nadir, 1500/mm³; range, 200–3600) and thrombocytopenia was observed in

23% (median platelet count nadir, 74,000/mm³; range, 38,000–120,000). Considering all courses of V-CAP, leukopenia occurred in 100% of patients (median wbc count nadir, 1500/mm³; 27% with ≤ 1000) and thrombocytopenia occurred in 48% (median platelet count nadir, 82,000/mm³; 22% with ≤ 50,000).

Renal toxicity (serum creatinine > 1.5 mg/dl) was observed in 22% of patients, but no patient developed a serum creatinine > 1.9 mg/dl. No clinically apparent neurologic or auditory toxicity was observed from cisplatin (highest total cumulative dose, 471 mg/m²).

DISCUSSION

The 46% regression rate (13 regressions among 28 patients) noted in this study of V-CAP was similar to the 50% regression rate (ten regressions among 20 patients) noted for CAP-II (2) in patients with advanced LGC of the lung and no prior chemotherapy exposure. The age, ECOG PS, sex ratio, etc, of the patients treated with CAP-II and V-CAP were quite similar. Median times to tumor progression for regressors were also quite similar (196 days for CAP-II versus 204 days for V-CAP) as were median survivals for all patients (173 days for CAP-II versus 211 days for V-CAP).

The myelosuppression for the current V-CAP regimen (VP-16-213, 50 mg/m²/day for 3 days) was essentially identical to that for the earlier V-CAP (5) regimen (VP-16-213, 125 mg/m² on Day 1). We believe the VP-16-213 dose to be at or very close to maximum for routine use on an outpatient basis.

V-CAP appears to be an effective regimen for the palliative treatment of patients with advanced LGC of the lung. This study did not attempt to define the role of VP-16-213 in that disease. Hopefully, a currently ongoing prospectively randomized trial comparing CAP versus V-CAP versus T-CAP (triazinate plus CAP) in patients with locally unresectable (M_0) disease will do so.

REFERENCES

1. Eagan RT, Ingle JN, Frytak S, et al. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat Rep 61:1339–1345, 1977.
2. Eagan RT, Frytak S, Creagan ET, et al. Phase II study of cyclophosphamide, adriamycin, and *cis*-dichlorodiammine-platinum(II) by infusion in patients with adenocarcinoma and large cell carcinoma of the lung. Cancer Treat Rep 63:1589–1591, 1979.
3. Eagan RT, Carr DT, Frytak S, et al. VP-16-213 versus polychemotherapy in patients with advanced small cell lung cancer. Cancer Treat Rep 60:949–951, 1976.
4. Eagan RT, Ingle JN, Creagan ET, et al. VP-16-213 chemotherapy for advanced squamous cell carcinoma and adenocarcinoma of the lung. Cancer Treat Rep 62:843–844, 1978.
5. Eagan RT, Creagan ET, Ingle JN, et al. VP-16, cyclophosphamide, adriamycin, and *cis*-platinum (V:CAP-I) in patients with metastatic adenocarcinoma of the lung. Tumori 65:105–109, 1979.

Working Conference on Neuroblastoma Treatment Trials [1]

Richard S. Ungerleider [2,*]

A working conference on neuroblastoma treatment trials took place on January 14, 1981, at the National Cancer Institute, Bethesda, Maryland. Neuroblastoma is a malignancy of infancy and early childhood, yet in contrast to Wilms' tumor and acute lymphoblastic leukemia, the outlook for most neuroblastoma patients remains poor. This conference was convened by the Division of Cancer Treatment of the National Cancer Institute to update current treatment results, to discuss a rational design of future studies, and to review pertinent biologic information regarding neuroblastoma.

The conference opened with a statement by **Ungerleider** (Bethesda) pointing out that therapeutic success in neuroblastoma remains elusive; with only 500 newly diagnosed cases yearly in the US, it seems critical that a consistent approach be taken by investigators involved in the search for effective therapy. A series of questions, concerned with the following aspects of the disease, was posed to the participants:

1. *Staging*—What is the significance of regional lymph node involvement in neuroblastoma? What is the significance of complete resectability? How is complete response defined?

2. *Biochemical markers*—Have catecholamine levels proved useful in predicting outcome? Do other markers provide information on prognosis?

3. *Cell kinetics and chemotherapy*—Can therapy based on the kinetics of neuroblasts be designed? Why use combination chemotherapy? How can the development of resistance be avoided? Is high-dose chemotherapy useful?

4. *Radiation therapy*—What is the role of conventional radiotherapy? Is total-body irradiation a useful modality in neuroblastoma? Can radiation be delivered in situ via radiolabeled immunoglobulin?

5. *Other approaches to therapy*—Can the unique biologic characteristics of neuroblastoma be exploited to provide for successful therapy?

The discussion of these questions constitutes the basis of this report.

STAGING

Evans presented the Children's Cancer Study Group (CCSG) staging system, and **Green** (Memphis) presented the system used at St. Jude Children's Research Hospital. A major difference between these systems resides in Green's classification of patients with regional lymph node involvement into the same stage as those with disseminated disease. Data were presented from St. Jude studies demonstrating that survival for patients with distant or regional disease was similar when patients with regional disease had demonstrable lymph node involvement.

Evans agreed that lymph node positivity is probably a significant variable, although it has been impractical to incorporate into the CCSG staging system. Small resectable primaries often have no nodes to sample, whereas CCSG stage III midline celiac axis tumors often have simple biopsy due to technical difficulties in sampling the nodes. **D'Angio** (Philadelphia) questioned whether the St. Jude node data differentiated between ipsilateral and contralateral nodes; it is his experience that positive contralateral nodes provide a worse prognosis than positive ipsilateral nodes, and that ipsilateral node patients may therefore be downstaged. Green replied that he had not analyzed for this variable. **Frantz** (Boston) pointed out that it is impossible to predict the involvement of a node from its intraoperative appearance, and that node sampling was mandatory. Furthermore, he maintained that pa-

[1] Included among the conference participants were representatives of a number of medical disciplines: A. Evans, C. Frantz, A. Green, A. Hayes, L. Helson, A. Mauer, D. Poplack, S. Siegel, and T. Vietti (pediatric oncology); G. D'Angio, L. Kun, and M. Wharam (radiotherapy); J. Grosfeld and I. Smith (surgery); G. Altschuler (pathology); and J. Bottenstein and G. Melino (immunology). Additional representatives of cancer centers and cooperative groups engaged in National Cancer Institute-supported trials in neuroblastoma were also in attendance.

[2] Pediatric Section, Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, MD.

**Reprint requests to*: Richard S. Ungerleider, MD, Pediatric Section, CTEP, DCT, NCI, Landow Bldg, Rm 4A12, National Institutes of Health, Bethesda, MD 20205.

tients with disseminated disease who undergo initial primary tumor resection have a better prognosis than those who have unresectable primary tumors. The International Union Against Cancer (UICC)/TNM staging system was then considered, with **Hayes** (Memphis) commenting that a system which implied a progressive tumor-node-lymphatics-hematogenous spread appeared to be inconsistent with what is observed in neuroblastoma. Evans expressed that she would have difficulty subjecting a patient to a second laparotomy for staging upon referral to her institution following resection elsewhere; Hayes believed it would be critical to enable the distinction between a child who needed no further therapy and one who needed potentially life-threatening chemotherapy. There was general agreement among the participants that lymph node status was important and that it is highly desirable for all patients to be evaluated for nodes at surgery when feasible. D'Angio pointed out that staging systems were temporary devices which ultimately depend on current therapy, and that it was proper to consider alterations in staging as new information was collected. A working party charged with establishing guidelines for staging neuroblastoma was appointed: J. Casper, A. Green, J. D'Angio, I. Smith, J. Grosfeld, R. Nitschke, and R. Ungerleider.

Green discussed the criteria for complete response at his institution, which consist of disappearance of all clinical indicators of disease but which exclude findings on second-look surgery. He felt it imperative to delay final assessment of responsiveness until 4 months from diagnosis in order to allow bone lesions to heal completely. His experience is that long-term survival hinges on reaching a clinical complete remission, but that presence of residual tumor did not necessarily decrease survival. **Siegel** (Los Angeles) pointed out that CCSG response criteria are similar, and that assessment is delayed until 22 weeks after diagnosis. **Vietti** (St. Louis) cautioned against disregarding earlier evidence of response even when the patient goes on to early relapse. **Mauer** (Memphis) commented that complete remission status confers survival advantage only when it is apparent at 4–5 months, as patients with rapid early response often go on to rapid early relapse. Participants agreed that it was crucial to select the time for assessment of complete response so that it identifies patients who have a survival advantage, and to compare maximum response at other times with this landmark.

BIOCHEMICAL MARKERS

Siegel discussed his experience monitoring catecholamine levels in urine of neuroblastoma patients, stressing the utility at diagnosis of the vanillylmandelic acid/homovanillic acid (VMA/HVA) ratio rather than absolute levels of these substances (a ratio > 1.5 indicates better prognosis independent of age and stage). He demonstrated the persistence of the biochemical phenotype at relapse and in the tissue culture line of a given patient. **Altschuler** (Oklahoma City) suggested using gas chromatographic analysis for catecholamines, but Siegel championed paper chromatography since it is simple and reproducible and since age-related normal data exist. Data pertaining to the use of carcinoembryonic antigen, $alpha_1$-antitrypsin, and haptoglobin levels did not indicate any advantage over simply monitoring catecholamines. Frantz has found serum LDH levels to be of prognostic import in stage IV, with levels twice normal associated with poor prognosis in a small number of patients. **Helson** (New York) has not found any advantage in monitoring urine cystathionine rather than VMA/HVA, but uses serum DOPA to identify active disease. Evans has found serum ferritin levels useful in identifying disease activity, and in distinguishing stage IV from stage IVs patients.

CELL KINETICS AND CHEMOTHERAPY

Hayes discussed design of chemotherapy schedules based on cell kinetics as pursued at her institution. She reviewed the rationale for utilizing cyclophosphamide as a recruiting agent prior to exposure to phase-specific doxorubicin, and showed how this approach has resulted in an increased complete remission rate in her patients. The total pattern of labeling index and mitotic index of bone marrow neuroblasts before therapy, after cyclophosphamide, and after doxorubicin was predictive of outcome using remission as the end point. The initial labeling index alone did not correlate with response, however. Labeling index measurements following low-dose cisplatin (DDP) administration suggested that this agent acts by synchronization in S phase rather than by recruitment; this, combined with clinical evidence that DDP prior to VM-26 produced higher response rates than the reverse (St. Jude and CCSG data) lent credence to the incorporation of DDP into front-line therapy for stage IV patients following cyclophosphamide for recruitment and prior to doxorubicin for S-phase cell killing. This approach also allows for the measurement of labeling indices after administration of each agent, which might then identify reasons for failure in a particular patient. This is preferable to the situation of all three drugs given simultaneously wherein failure does not allow for assessment of the relative contribution of each agent. Hayes emphasized that

this type of study can be done on a collaborative basis as St. Jude has been doing with investigators in Milwaukee and Dallas, with samples transferred by air express.

Hayes went on to address emergence of resistance, and suggested that it may be unavoidable. She felt that labeling indices and the ability of neuroblasts to grow in culture could be used to predict poor response, indicating that a more favorable situation exists at relapse if the patient's labeling index is < 10% and the cells are unable to grow in culture. A high labeling index and successful growth in culture would suggest that alteration in therapy is necessary.

Helson discussed kinetics of human neuroblastoma carried in nude mice and indicated that surgical removal of half the tumor mass leads to an increased S phase at 24 hours. This may have implications for scheduling chemotherapy following partial debulking. He has also embarked on a phase II study of melphalan plus dianhydrogalactitol based on his mice studies, and feels that the early evidence is promising. Mauer cautioned that response criteria be objective and strictly defined. Siegel noted that melphalan alone would probably not represent an advantage over cyclophosphamide as both are alkylators. Helson felt that higher doses of alkylators might produce increased responses, while **Poplack** (Bethesda) noted that escalating doses of cyclophosphamide as high as 200 mg/kg in association with vincristine and DTIC did not produce the anticipated responses but resulted in significant toxicity. Siegel and Mauer agreed that high-dose cyclophosphamide did not represent a fruitful area for further investigation.

Frantz presented data suggesting improved disease-free survival of CCSG stage IV patients in whom the primary tumor was resected compared to those in whom it was unresectable. All patients in whom computerized tomographic scan suggested a remote possibility of resection underwent exploration. Of 20 stage IV patients > 1 year of age at diagnosis, seven had > 90% of the primary tumor resected. All patients received six-drug combination chemotherapy and responders underwent a delayed or second laparotomy in an attempt to resect residual tumor; this was followed by radiation to the primary tumor bed and then by continued chemotherapy. Both rate of response (as defined by Green) and duration of disease-free survival were significantly greater in patients with successful resection. It is unclear whether reduction of tumor bulk improves outcome or whether resectability is a "marker" of biologically different disease. **Grosfeld** (Indianapolis) and Siegel pointed out that 14 stage IV patients who underwent initial primary resection were entered in CCSG studies; they had a short (6.5-month) median survival which was not better than those who did not have resection. Frantz suggested the difference in results might be due to the different chemotherapy.

RADIATION THERAPY

In discussing the role of conventional radiation therapy, D'Angio reviewed CCSG material supporting his contention that stage I patients need no further therapy beyond resection and discussed Children's Hospital of Philadelphia experience indicating that X-ray therapy after resection does not improve outcome for CCSG stage II. It is his practice to irradiate stage III patients although there has been no prospective randomized trial to demonstrate that the addition of radiotherapy is superior to no radiotherapy. He has been investigating fractionated low-dose total body irradiation (TBI) in association with vincristine, DTIC, and cyclophosphamide, delivering 50 rad two times weekly at 3–4-week intervals in order to capitalize on the slightly increased survival capacity of marrow cells compared with neuroblasts when exposed to radiation and to allow time for repair. It is too early to analyze for efficacy. Profound and sustained thrombocytopenia has been noted at the 400–500-rad mark. He has also been using TBI in conjunction with VM-26, doxorubicin, and melphalan followed by allogeneic or autologous bone marrow rescue in a small number of patients.

Kun (Milwaukee) reported his experience with fractionated low-dose TBI used as maintenance following a 4-month term of therapy with sequential cyclophosphamide and doxorubicin plus laparotomy to remove residual tumor in patients presenting with disseminated disease. TBI was delivered as 10-rad fractions five times weekly to a total of 100–120 rad, while 2400 rad were delivered to the primary if it proved unresectable. His conclusion after treating seven patients was that this regimen did not appear effective, and he has subsequently abandoned it. A concurrent maintenance program at St. Jude Children's Research Hospital which omitted TBI but randomized between immunotherapy with BCG and chemotherapy resulted in equally poor response.

Helson utilized hemibody irradiation (800 rad over 4 days to each half) following multiagent chemotherapy (including escalating doses of cyclophosphamide) and showed no advantage over previous Memorial Sloan-Kettering Cancer Center studies. **Wharam** (Baltimore) has treated four patients with TBI followed by autologous rescue and has had one long-term (3-year) survivor.

Wharam went on to discuss treatment of neuro-

blastoma with radiolabeled antiferritin. The rationale is based on the success of interstitial radiotherapy implants and radioactive iodine in other malignancies. Polyclonal antibody to ferritin is raised in rabbits, tagged with a radionuclide, and injected iv on a one-time-only basis to avoid sensitivity. As ferritin is produced by neuroblastoma cells, it is hoped that the isotope will be sequestered preferentially in the tumor. This program is in its early stages, and while he has found it feasible to deliver radiation in this manner without toxicity, the delivered dose is roughly 20% of that considered optimal.

The discussion continued with observations on the role of radiotherapy in neuroblastoma. Kun commented that systemic irradiation might best be delivered in conjunction with autologous marrow reinfusion when the marrow was known to be clear of tumor cells. This implies using St. Jude stage IIIA patients, or waiting until technology is available for clearing the marrow with reagents such as monoclonal antibodies or 6-hydroxydopamine prior to infusion. Hayes suggested that a prospective randomized trial might be done to ascertain whether conventional radiotherapy adds anything to the treatment of nonresectable local disease. In a nonrandomized study at St. Jude Children's Research Hospital, 16 such children received sequential cyclophosphamide and doxorubicin, five of whom were irradiated as well. Fourteen of the 16 children survive free of disease, including all 11 who had chemotherapy alone.

OTHER APPROACHES TO THERAPY

Bottenstein (Los Angeles) spoke about her studies elucidating the basic requirements of neuroblastoma growing in culture, as well as the production of autostimulatory growth factors, with the expectation that denying critical requirements in vivo might provide a new approach toward perturbing neuroblastoma in patients. Her studies indicate that transferrin is the most important requirement, followed by insulin, and she speculated that transferrin receptors on neuroblastoma cells might be an appropriate target for transferrin linked to cellular toxins such as ricin or diphtheria fragment A.

Melino (London) described a treatment regimen under development at Westminster Medical School in which neuroblastoma cells from the child's tumor are injected into the parent followed by plasmapheresis of the parent donor and separation of serum immunoglobulin. The immunoglobulin is chemically coupled to chlorambucil or daunomycin and infused into the patient with the hope that the chemotherapeutic agent is delivered preferentially to tumor cells. There is no attempt to absorb out non-neuroblastoma antibody, nor is the approximately 15% unbound drug removed from the solution. There was some concern among the participants relating to the specificity of the immunoglobulin for tumor cells, and to the relative contributions of bound and free drug. This regimen is currently in early clinical trial and is well-tolerated.

Helson then discussed the possibility of exploiting the ability of neuroblastoma cells to differentiate. He displayed photomicrographs of neuroblastoma cells in culture which showed morphologic evidence of differentiation following papaverine treatment, and in vivo maturation of neuroblastoma to ganglioneuroblastoma in a mouse. He reviewed the results of studies at his institution which utilized papaverine along with multiagent chemotherapy. Siegel commented that a phase II study of papaverine as a single agent might provide useful information. D'Angio questioned whether papaverine caused lysis of neuroblasts and thereby allowed adjacent ganglioneuroblasts to grow, or whether differentiation was truly induced. Siegel commented that chemotherapeutic regimens without specific differentiation inducers have likewise been associated with evidence of differentiation at second-look surgery.

DISCUSSION AND CONCLUSIONS

There then ensued a general discussion of how the basic approach to this disease might be improved. Hayes maintained that increased survival is an inappropriate end point at this time, and that the focus should be on seeking ways to improve the complete remission rate as the first step in ultimately extending survival. She emphasized the need for employing a staging system that sorts out children who need only surgery, children who respond to current therapy, and children who need an entirely new approach. She questioned how one might analyze the relative contributions of the various agents in a multidrug multimodal regimen, and stressed the need to rationally build upon previous combinations. Altschuler suggested that investigators be more specific about the pathologic appraisal of tumor, and it was pointed out that Chatten at Children's Hospital of Philadelphia was actively cataloguing the histolopathologic findings in all newly diagnosed patients. Mauer emphasized both the need for new agents and the proper conduct of phase II studies such that no active compound is erroneously discarded. Grosfeld suggested considering the nutritional status of the patient at diagnosis as a possible staging criterion, as he finds that well-nourished patients have a much better 1-year survival

than do the poorly nourished. Helson commented that he has found the anorexia-inducing agent cholecystikinase in the serum of five patients, which may relate the biologic behavior of the tumor to the development of malnutrition. Siegel felt that the biologically unique features of neuroblastoma may well provide ideas for future therapy. D'Angio expressed the need for an orderly systematic examination of the role of preoperative and postoperative radiotherapy. The participants agreed with the concept that neuroblastoma is not a monolithic illness but rather a heterogeneous disease.

Ungerleider summarized the conclusions of the conference as follows:

1. For proper evaluation of studies, reported information must include the criteria for response, the time of assessment for response, the staging system utilized, and the previous therapy given the patient.

2. A working party has been charged with exploring the standardization of staging, and the possible incorporation of surgical resectability, lymph node status, biochemical markers (including catechols, ferritin, plasma DOPA, LDH), and computerized tomographic scan information. Previously collected information concerning lymph node status will be reanalyzed.

3. Chemotherapy studies should be directed toward increasing the proportion of patients reaching complete remission. Treatment regimens should be constructed rationally, based on cell kinetic, pharmacologic, biologic, or other principles, and should provide opportunity for the analysis of reasons for failure. Re-emphasis should be placed on the evaluation of new agents.

4. Radiotherapy studies should systematically evaluate the role of irradiation in treatment of residual disease. Total-body irradiation, hemibody irradiation, and radiolabeled immunoglobulin remain procedures best pursued in pilot studies.

5. The unique biologic characteristics of neuroblastoma remain a potential source of effective therapy, and efforts to understand these processes should be encouraged.

Anaphylactic Reaction to Methotrexate Used in High Doses as an Adjuvant Treatment of Osteogenic Sarcoma

Recently, methotrexate (MTX) in high doses has been used in the treatment of several types of malignant tumors such as osteosarcoma, soft tissue sarcoma, breast cancer, and histiocytic lymphoma. In addition to the known adverse effects, there are few reported cases of anaphylactic reactions to high-dose MTX (1,2), and we report an additional two cases.

CASE REPORTS

Patient 1

Patient 1, a 39-year-old white woman with osteogenic sarcoma, was treated according to the adjuvant program of the National Cancer Institute of Canada. She was admitted for her seventh treatment with high-dose MTX; after hydration and urine alkalinization, she started receiving 12 g of MTX in 2 liters of iv fluid to be infused over 6 hours. Within 10 minutes she developed an urticarial rash on both forearms. The MTX was stopped, and the patient received 50 mg of Atarax im followed by 50 mg every 4 hours for eight doses. The MTX was then resumed, and after the infusion of about 5 g of MTX, she developed nausea, vomiting, and urticarial rash. Three weeks later she was admitted for another course of high-dose MTX. She was premedicated with 50 mg of Benadryl iv and 50 mg of hydrocortisone iv, and the MTX infusion was started. After a few minutes she again developed generalized urticarial rash and the therapy had to be stopped. In this patient, two different preparations of MTX had been used, one from Lederle Laboratories and the second from the National Cancer Institute (Bethesda, MD); both produced allergic reactions. The second preparation of MTX had no preservative.

Patient 2

Patient 2, a 34-year-old woman, started receiving treatment with high-dose MTX according to the National Adjuvant Protocol of Canada following amputation of her left leg for primary osteogenic sarcoma. Within a few minutes from the start of her second treatment with 12 g of MTX to be infused over 6 hours, she developed urticarial rash on her abdomen and both forearms, together with facial edema. She was immediately given 200 mg of hydrocortisone iv, and the MTX infusion was continued in combination with 200 mg of hydrocortisone every 6 hours. The National Cancer Institute preparation, which does not contain preservative, was used.

DISCUSSION

In the first report of two cases, anaphylactic reaction to high-dose MTX (1) was thought to be due to pretreatment with BCG. The authors suggested that BCG, through its immunologic effects, may play a role in the development of an immune reaction to MTX or contaminants of the preparation. Other investigators (2) demonstrated that the reaction can develop without any prior immunotherapy and this was supported by our own observations. Our experience is in keeping with that of the second investigators, that MTX alone, without the preservative benzyl alcohol, can also produce an anaphylactic reaction.

In our two patients, no desensitization was attempted. The first patient was at the end of her treatment schedule. The second patient was treated with doxorubicin, which may be equally effective. In addition, it was unlikely that desensitization would have prevented an anaphylactic reaction (2).

REFERENCES

1. Goldberg NH, Romolo JL, Austin EH, et al. Anaphylactoid type reactions in two patients receiving high dose iv MTX. Cancer 41:52–55, 1978.
2. Gluck-Kuyt I, and Irwin LE. Anaphylactic reaction to high-dose MTX. Cancer Treat Rep 63:797–798, 1979.

P. Klimo
E. Ibrahim[1]

Cancer Control Agency of British Columbia
#700 – 686 W Broadway
Vancouver, British Columbia, Canada V5Z 1G1

April 16, 1980

[1]*Present address:* Department of Oncology, King Faisal University, Dammam, Saudi Arabia.

Baker's Antifol in Children With Therapy-Resistant Solid Tumors: A Southwest Oncology Group Study[1]

Baker's antifol (BAF), or triazinate, is a triazine

[1]Supported in part by Public Health Service grants CA-11233, CA-16388, CA-05587, CA-26756, CA-20549, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

TABLE 1.—Summary of BAF therapy in 10 children

Tumor	BAF therapy			
	No. of courses	Dose (mg/m^2/day)	Duration of therapy	Response *
Neuroblastoma	1	100	20†	NR
Neuroblastoma	1	250	21	INCD
Neuroblastoma	1	100	16	INCD
Wilms' tumor	1	250	20†	INCD
Rhabdomyosarcoma	1	250	14	INCD
Ewing's sarcoma	1	250	21	INCD
Hodgkin's disease	1	250	56	NR
Embryonal cell carcinoma	1	250	37	INCD
Osteogenic sarcoma	2	250/100	85	INCD
Ependymoma	9	250	222	INCD

*NR = no response; INCD = increased disease.
†Patient died.

folate antagonist that inhibits dihydrofolate reductase. In adults it was effective against lung adenocarcinoma, colorectal carcinoma, and renal carcinoma (1). We demonstrated that the drug was not effective in children with late-stage acute lymphocytic leukemia (2). This report is concerned with the effectiveness of BAF in children with far-advanced solid tumors.

METHODS

Patients with solid tumors were eligible for study if they (*a*) were < 18 years old at the time of diagnosis and < 21 years old at the time of entry in the study, (*b*) had at least one measurable lesion not recently treated with radiation therapy, (*c*) were resistant to conventional therapy, (*d*) had not received chemotherapy for 1 week and had never received methotrexate, (*e*) did not show any significant toxic effects from previous therapy, and (*f*) had an SGOT < 60 milliunits/ml and/or bilirubin < 1.5 mg/dl and/or creatinine < 1.5 mg/dl. Informed consent was obtained according to the guidelines for each institution.

BAF (250 mg/m^2) was infused for 60 minutes/day × 3 days in patients with normal liver and kidney functions. If SGOT was between 40 and 60 milliunits/ml and/or if bilirubin and serum creatinine were between 1.1 and 1.5 mg/dl, BAF was reduced to 100 mg/m^2/day × 3 days. The courses were repeated every 3 weeks. Physical examination and total wbc counts were required weekly. Liver and kidney function tests were required before each new course of therapy. The response to BAF therapy was evaluated according to the criteria for solid tumors developed by the Pediatric Division of the Southwest Oncology Group (3).

RESULTS AND DISCUSSION

Ten patients were entered in the study. Their ages ranged between 1 9/12 and 14 6/12 years, with a median of 8 10/12 years. The type of tumors, duration of therapy, number of courses, and BAF doses given to the individual patients are summarized in table 1. No patients responded to therapy.

The main toxic reactions observed were erythematous rashes with vesiculation (two patients) and mucositis with ulceration (one). Moderate vomiting and diarrhea were noted in two patients. Two children developed anemia (hemoglobin < 10 g/dl) and thrombocytopenia (platelets < 100,000/μl). Neutropenia was not noted.

During treatment with BAF, eight of ten patients with childhood solid tumors showed progressive disease, and the other two did not respond to therapy. The study was terminated since the drug was ineffective in these patients and since effectiveness has only been observed in patients with carcinoma and not in patients with sarcomas (1).

REFERENCES

1. RODRIGUEZ V, RICHMAN SP, BENJAMIN RS, ET AL. Phase 2 study with Baker's antifol in solid tumors. Cancer Res 37:980–983, 1977.
2. NITSCHKE R, VIETTI T, RAGAB A, ET AL. Baker's antifol in children with late-stage acute lymphocytic leukemia. Cancer Treat Rep 62:2109–2110, 1978.

3. Nitschke R, Starling KA, Vats T, et al. *Cis*-diammine-dichloroplatinum (NSC-119875) in childhood malignancies. Med Pediatr Oncol 4:127–132, 1978.

R. Nitschke*
S. K. Morgan
V. J. Land

Southwest Oncology Group[2]

August 14, 1980

[2]This study was conducted by Southwest Oncology Group members at the following locations: Oklahoma Children's Memorial Hospital, Oklahoma City (*R. Nitschke*); Medical University of South Carolina, Charleston (*S. K. Morgan*); and Washington University, St. Louis, MO (*V. J. Land*).

**Reprint requests to:* Southwest Oncology Group Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, KS 66103.

Pulmonary Toxicity Studies of Bleomycin Tested Before and After Its Expiration Date

This letter addresses the relative pulmonary toxicity of biologically active bleomycin (1).[1] Most pulmonary toxicity studies carried out in animals (1–3) use bleomycin[2] that has passed its expiration date, whereas clinical studies use unexpired bleomycin. The issue of relative pulmonary toxicity is of some importance considering the possible impact of animal studies on the present and future clinical investigations using bleomycin and its analogs.

The present study was performed between February and April 1979 using two lots of bleomycin, one expired and the other unexpired.[3] Lot J8X02 (unexpired) had an expiration date of December 30, 1979, > 8 months after the completion of the study. Lot E6X01 (expired) had an expiration date of May 1977 and was selected because it was the oldest sample of expired bleomycin which demonstrated acceptable antibacterial activity.[1] Both lots of bleomycin were stored in ampules at room temperature and samples for dosing mice were prepared in buffered saline immediately before use.

BDF_1 male mice weighing 22 ± 2 g were randomly allocated into five separate groups of 33 mice each. Each group of mice was injected sc twice weekly (Tuesday and Friday) for 4 weeks with either saline, bleomycin (lot J8X02) at a dose of 8.9 or 17.9 units/kg, or bleomycin (lot E8X01) at a dose of 8.9 or 17.9 units/kg (17.9 units = 10.0 mg). Previous experiments had demonstrated that bleomycin at 8.9 and 17.9 units/kg produced significant dose-related increases in lung hydroxyproline in mice (3).

Approximately ten mice from each group were killed at the end of 6, 8½, and 12 weeks (2, 4½, and 8 weeks after termination of treatment, respectively). Individual mouse lungs were assayed for hydroxyproline by the method of Woessner (4).

Both lots of bleomycin produced a dose-related increase in lung hydroxyproline content at the doses used. There were no statistically significant differences between the two lots in terms of total hydroxyproline content, dose-response relationship, or survival. There was significantly less lung hydroxyproline in control mice than in the mice receiving the lowest dose (8.9 units/kg) of expired or unexpired bleomycin. Moreover, histopathologic evaluation performed at 12 weeks demonstrated that both lots when given at 17.9 units/kg produced typical bleomycin pulmonary lesions, ie, interstitial pneumonitis, fibrosis, alveolitis, proteinosis, and alveolar macrophages.

Both lots of bleomycin were within specification for biologic activity as indicated by the standard antibacterial assay. Furthermore, equivalent doses of both lots of bleomycin produced equivalent patterns of weight change in mice.

The present study demonstrates that expired and unexpired bleomycin produced equivalent degrees of pulmonary toxicity in mice. The lots of bleomycin used in this study were selected to maximize the age difference but to have equivalent biologic activities, thus avoiding the possibility that a loss in biologic activity may mask a relative change in pulmonary toxicity. Inasmuch as the maximum age difference which might be encountered in the clinic is 18 months, the results of this study suggest that it is highly unlikely that significant differences in clinical pulmonary toxicity will be encountered due to variations in the age of the bleomycin employed.

[1]Blenoxane, new drug application, vol 1, 1971.

[2]Requests for bleomycin by investigators doing animal studies are routinely filled with returned goods, expired bleomycin.

[3]The clinical dose formulation of bleomycin has an official expiration date of 18 months following manufacture. Unexpired and expired represent material prior to and after expiration date, respectively.

REFERENCES

1. Crooke ST, and Brander WT. Bleomycin, a review. J Med 7:333–427, 1976.
2. Sikic BI, Young DM, Mimmaugh EG, et al. Quantification of bleomycin pulmonary toxicity in mice by changes in lung hydroxyproline content and morphometric histopathology. Cancer Res 38:782–792, 1978.

3. SCHLEIN A, SCHURIG JE, BACA C, ET AL. Pulmonary toxicity studies of bleomycin and talisomycin. Cancer Treat Rep 65:291–297, 1981.
4. WOESSNER JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of the amino acid. Arch Biochem Biophys 93:440–447, 1961.

Abraham Schlein
John E. Schurig
Charles D. Baca
William T. Bradner
Stanley T. Crooke[4]

Bristol Laboratories
PO Box 657
Syracuse, NY 13201

September 15, 1980

[4]We thank Dr. R. Hirth for the histopathologic evaluation.

Effect of Tamoxifen in Premenopausal Japanese Women With Advanced Breast Cancer[1]

An antiestrogenic drug, tamoxifen, has been recently introduced as a hormonal measure for the treatment of breast cancer and has provided promising results in several trials carried out for postmenopausal patients in Europe and the US.

The incidence of breast cancer is much lower and the prognosis is much better in Japan than in European countries (1). Because the difference is considered to be largely related to differences in hormonal circumstances, it seemed important to evaluate the effects of hormonal therapy with tamoxifen in Japanese patients.

In the following report, we present recent results of tamoxifen therapy in our premenopausal patients with advanced breast cancer.

MATERIALS AND METHODS

Twenty-one of the 45 patients who were entered in the protocol during the past 3 years were premenopausal; 16 of the 21 had recurrent breast cancer and five had primary advanced breast cancer. All of the patients had objectively measurable lesions, and ten were studied for estrogen receptor (ER) by the dextran-coated charcoal method. Eight patients had had prior therapies, six with chemotherapy and two with surgical endocrine therapy, but none had received any anticancer therapy within 1 month before the start of treatment. Tamoxifen was administered each day orally at a dose of 20–40 mg/day (10–20 mg twice a day).

[1]Supported in part by a Grant-in-Aid for Cancer Research (54–3) from the Ministry of Health and Welfare.

RESULTS

The overall rate of response (complete and partial responses) to tamoxifen was 38%, as shown in table 1. The interval between the start of therapy and the onset of partial response was 4–14 weeks, with a mean of 7.4 weeks. The dose of tamoxifen given during this period was 560–2080 mg, with a mean of 1056 mg. Duration of response was 10–76 weeks, and in three of the patients the response is continuing. The total dose of the drug was 1680–31,450 mg for the responders and 620–1180 mg for the nonresponders.

Soft tissue lesions responded more favorably to tamoxifen than bone and visceral lesions.

In ten premenopausal patients studied for ER, the four patients who were ER-positive responded to tamoxifen, while none of six who were ER-negative responded. Because the number of patients tested for ER was small, the relationship between ER positivity and response rate was not conclusive.

As side effects of tamoxifen, gastrointestinal problems such as nausea and vomiting were observed in three patients but were usually mild. Amenorrhea occurred in two of the 21 premenopausal patients. The doses of tamoxifen given before the onset of amenorrhea were 1400 and 1680 mg, respectively. No other symptoms such as bone marrow suppression, hepatic and renal disorders, or hypercalcemia were observed.

DISCUSSION

Cole et al (2) and Kiang and Kennedy (3) have reported overall response rates of 22% and 32%,

TABLE 1.—Effect of tamoxifen according to dominant sites of lesions

Site of lesion	Responders (CR, PR) *	Nonresponders (NC, PD) *
Soft tissue †	8 (53%)	7 (47%)
Bone	0	2
Viscera	0	4
Total	8 (38%)	13 (62%)

*Criteria of the International Union Against Cancer; CR = complete response, PR = partial response, NC = no change, PD = disease progression.
†Including locally advanced primary breast cancer.

respectively, for tamoxifen used in the treatment of advanced breast cancer in European patients, most of whom were postmenopausal. The response rate in our series was 29%, which is in the same range. However, the result obtained for the present premenopausal patients (38%) was even higher than the result for the postmenopausal patients (21%). This finding seems contradictory to the few reports (4) available on the effect of tamoxifen in premenopausal patients. However, the eight responders among our premenopausal patients had soft tissue lesions (four primary breast tumors, three in the lymph nodes, and one in a local skin recurrence); none of the responders had had prior therapy. These factors may relate to the relatively high response rate in our series.

REFERENCES

1. NEMOTO T, TOMINAGA T, CHAMBERLAIN A, ET AL. Differences in breast cancer between Japan and the United States. JNCI 58:193–197, 1977.
2. COLE MP, JONES CTA, and TODD IDH. A new anti-estrogenic agent in late breast cancer. Br J Cancer 25:270–275, 1971.
3. KIANG DT, and KENNEDY BJ. Tamoxifen (antiestrogen) therapy in advanced breast cancer. Ann Intern Med 87:687–690, 1977.
4. HEUSON JC. Current overview of EORTC clinical trials with tamoxifen. Cancer Treat Rep 60:1463–1466, 1976.

Tomio Wada*
Hiroki Koyama
Toshio Terasawa

Department of Surgery
The Center for Adult Diseases, Osaka
1-3-3, Nakamichi, Higashinari-Ku
Osaka 537, Japan

November 13, 1980

Double Alkylator Therapy (Thiotepa Plus Chlorambucil) for Previously Treated Advanced Ovarian Cancer

Combination chemotherapy for patients with advanced ovarian cancer has resulted in response rates between 60% and 80% (1). The treatment of relapsed patients or patients not responding to initial chemotherapy, however, has produced responses in only 6%–10% (2). Since primary chemotherapy regimens for ovarian cancer contain an alkylating agent, most second-line regimens have not included such agents because of the traditional belief that resistance to one alkylating agent implies cross-resistance with other alkylators. However, clinical studies in multiple myeloma (3) and Hodgkin's disease (4) and experimental animal studies (5) suggest that resistance to a specific alkylating agent does not always result in resistance to other alkylators and that alkylators can have synergistic effects.

We therefore treated 18 patients with advanced ovarian cancer refractory to initial chemotherapy with a combination of alkylators: thiotepa, an aziridine derivative, together with chlorambucil, a mechlorethamine analog. Both agents have similar response rates (50%–64%) in previously untreated patients. Because patients had received extensive previous treatment, both drugs were administered at a reduced dose for the initial cycle: thiotepa at a dose of 0.2 mg/kg iv on Days 1–3 and chlorambucil at a dose of 0.2 mg/kg orally on Days 1–14, with no therapy on Days 15–28. Dose escalations were required: chlorambucil was increased by 1.0 mg/day and thiotepa was increased by 0.1 mg/kg/day if the wbc count remained $> 2500/mm^3$ during the preceding cycle.

There were no instances of objective response in the 18 patients with refractory advanced ovarian cancer treated with thiotepa plus chlorambucil. From this sample size, there is a $< 5\%$ probability that a 15% response rate exists for thiotepa plus chlorambucil in this patient population. A median of three cycles (range, one to six) of thiotepa and chlorambucil were administered before disease progression occurred.

The major toxic effect of thiotepa and chlorambucil in these patients, all of whom had had extensive prior chemotherapy, was myelosuppression. Six of the seven patients who received three or more cycles of chemotherapy required at least a 25% dose reduction because of wbc counts $< 2000/mm^3$ or platelet counts $< 100,000/mm^3$.

It is still possible that alkylating agent synergism plays a role in the treatment of patients with ovarian cancer and may be, in part, a reason for the increased initial response rates observed with newer combinations, such as Hexa-CAF and Chex-UP. These combinations include cyclophosphamide as well as at least one other drug whose mechanism of cytotoxicity may be related to alkylation (eg, hexamethylmelamine, cisplatin). It is possible that relapsed patients have already developed resistance to the potential synergistic effects of alkylators (which were part of their initial chemotherapy regimens) and this may account for the lack of therapeutic benefit observed with thiotepa and chlorambucil in patients with refractory ovarian cancer.

REFERENCES

1. YOUNG RC. *In* Gynecologic Malignancies in Cancer Chemotherapy (Pinedo HM, ed), 1980, pp 32–350.
2. STANHOPE RC, SMITH JP, and RUTLEDGE F. Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
3. BERSAGEL DE. Treatment of plasma cell myeloma with cytotoxic agents. Arch Intern Med 135:172, 1975.
4. DOAN CA, WISEMAN BK, and BOURONCLE BA. Clinical evaluation of CB 1348 in leukemias and lymphomas. Ann NY Acad Sci 68:975–995, 1958.
5. SCHABEL FM. New experimental drug combinations with potential clinical utility. Biochem Pharmacol Suppl 2:163–176, 1974.

Robert F. Ozols*
Diane M. Howser
Robert C. Young

Medicine Branch
Clinical Oncology Program
Division of Cancer Treatment
National Cancer Institute
Bethesda, MD 20205

April 27, 1981

*Reprint requests to: Robert F. Ozols, MD, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, Bethesda, MD 20205.

Phase II Study of Cyclophosphamide and Hexamethylmelamine in Non-Oat Cell Bronchogenic Carcinoma[1]

Between May and December 1978, 25 patients (22 males and three females) with measurable, nonresectable, and histologically proved non-oat cell

[1]Supported in part by a generous contribution from the Italian Association for Cancer Research, Milan.

TABLE 1.—Response and survival according to stage

Response	No. of patients				Median survival (wks)
	Total	Limited disease	Extensive disease		
			M_0	M_1	
Stable disease	10	5	4	1	40
Progression	10	3	4	3	35
Median survival (wks)	37	30	38		

bronchogenic carcinoma were entered in this study. Their ages ranged from 36 to 76 years (mean, 61). All patients had stage II or III disease; nine had limited disease and 16 had extensive disease (11 M_0 and five M_1). Cell types were: epidermoid carcinoma (20 patients), adenocarcinoma (three), and large cell carcinoma (two). All patients had a Karnofsky performance status > 50, and none had had prior chemotherapy or radiotherapy. Eligibility criteria were: wbc count > 4000/mm^3, platelet count > 100,000/mm^3, and normal cardiac, hepatic, and renal functions.

The chemotherapy schedule consisted of cyclophosphamide at a dose of 1 g/m^2 iv on Day 1 and hexamethylmelamine at a dose of 100 mg/m^2 orally on Days 1–14, every 3 weeks. After three cycles of chemotherapy, the patients were evaluated for response. Treatment was stopped in patients with disease progression; patients with stable disease received the same drugs on a maintenance schedule providing the same dose in 2-month cycles.

Twenty patients were evaluable for response after three cycles of chemotherapy; of the other five patients, four died during therapy and one discontinued chemotherapy because of a serious infectious episode. None of the 20 evaluable patients achieved an objective response. Ten patients had stable disease and ten had obvious disease progression. Response and survival are reported in table 1. The median survival time, determined according to Kaplan-Meier analysis (1), was 37 weeks; it was 35 weeks for patients with disease progression and 40 weeks for patients with stable disease. Patients with extensive disease had a longer survival than patients with limited disease. Only one patient had treatment discontinued because of vomiting and severe nausea.

This protocol failed to achieve any objective response, though median survival was longer than that reported with CCNU and doxorubicin (2) or with cyclophosphamide, doxorubicin, methotrexate, and procarbazine (3) and was in the same range as that reported for bleomycin, vincristine, and methotrexate (4).

REFERENCES

1. KAPLAN EL, and MEIER P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
2. TROWBRIDGE RC, KENNEDY BJ, and VOSIKA GJ. CCNU-Adriamycin therapy in bronchogenic carcinoma. Cancer 41:1704–1709, 1978.
3. VOGELZANG NJ, BONOMI PD, ROSSOF AH, ET AL. Cyclophosphamide, adriamycin, methotrexate, and procarbazine (CAMP) treatment of non-oat cell bronchogenic carcinoma. Cancer Treat Rep 62:1595–1597, 1978.
4. LIBRETTI A, PIAZZA E, SCANNI A, ET AL. Bleomycin, vincristine, and methotrexate (BOM) in squamous cell carcinoma of the lung: a phase II study. Cancer Treat Rep 65:171–172, 1981.

Maria Brambilla Pisoni
Arnaldo Libretti
Elena Piazza
Giuseppe Vago
Roberto Scapaticci
Celso Bianchi
Maurizio Tomirotti
Alberto Scanni
Maria Pia Calzavara
Lucia Cassani
Giorgio Scarpazza
Silvia Marsoni
Luciano Morasca[2,*]

May 12, 1981

[2]This study was done by the North Milan Cooperative Group.

*Reprint requests to: Luciano Morasca, MD, North Milan Cooperative Group, Mario Negri Institute for Pharmacological Research, Via Eritrea 62, 20157 Milan, Italy.

Sequential Combination Chemotherapy for Disseminated Melanoma: A Southwest Oncology Group Study[1]

The proliferation kinetics of human malignant melanoma have been described (1,2). The median

[1]Supported in part by Public Health Service grants CA-03096, CA-12644, CA-04920, CA-12014, CA-16943, and CA-17701 (National Cancer Institute) and RR-73 (General Clinical Research Centers Program of the Division of Research Resources) from the National Institutes of Health, Department of Health and Human Services.

cell cycle time (T_c) of human melanoma cells in vivo is about 3 days. Barranco and Humphrey (3) have shown that at low doses, bleomycin reversibly inhibits cell cycle progression at the S-G_2 boundary of the cell cycle. In vivo data indicate a 1.5- to 4-fold increase in the fraction of cells in the S phase 2–3 days following a single bleomycin synchrony treatment (4). Therefore, a protocol was developed directed by these proliferation kinetics of human melanoma and the potentially synchronizing effect of bleomycin in an attempt to improve these response rates.

MATERIALS AND METHODS

Sixty-one patients with disseminated melanoma who had not received previous chemotherapy were entered in the study. Four patients were not evaluable because of major protocol violations and inadequate data. Objective kinetic measurements were not performed on the individual patients. Fifty-seven patients were evaluable for response and all 61 patients were evaluable for toxic effects.

Bleomycin was given at a dose of 25 units/24 hours by constant iv infusion on Days 1–4. After a rest period from Days 5–7, BCNU (100 mg/m² × 2, with 4 hours between doses) was administered on Day 8. Two hours following the last dose of BCNU, DTIC was begun at a dose of 200 mg/m² iv daily for 5 days.

The second bleomycin infusion was begun on Day 23. After a rest period from Day 27 to 29, hydroxyurea was begun on Day 30 at a dose of 2000 mg/m²/day orally for 5 days.

The third bleomycin infusion was begun on Day 45. After a rest period from Day 49 to Day 51, dactinomycin at a dose of 0.2 mg/m² every 4 hours for two doses was given on Day 52. After a rest period on Days 53 and 54, a similar dose of dactinomycin was administered on Day 55.

Patients achieving a complete or partial response were then maintained on the combination of BCNU, hydroxyurea, and DTIC every 4 weeks as previously reported (5).

RESULTS

The rate of complete and partial remissions was 21% (with 95% confidence limits, 11.4%–33.9%). Table 1 gives the response rate by metastatic site(s) and performance status. The degree of toxicity encountered during therapy is also noted.

Three of the 12 responses observed were complete. The median survival of all 57 evaluable patients was 22 weeks.

TABLE 1.—Response to sequential chemotherapy for disseminated melanoma

Metastatic site and performance status	No. of patients responding/ No. in group (%) *
Metastatic site(s)	
Brain	2/8 (25)
Visceral	5/26 (19)
Lymph nodes only	3/3 (100)
Predominantly cutaneous	2/11 (18)
Lymph nodes and cutaneous	0/9
Total	12/57 (21)
Performance status (Karnofsky)	
≥ 70	10/49 (20)
50–70	2/8 (25)
Total	12/57 (21)
Degree of toxicity	**No. of patients ***
None or mild	19 (31)
Moderate	12 (20)
Severe	21 (34)
Life-threatening	9 (15)

* 57 patients were evaluable for response, and all 61 were evaluable for toxic effects.

Although nine patients experienced life-threatening toxic effects, no toxic deaths were noted (table 1). Seven patients had pulmonary symptoms, changes in physical examination of the lungs, or changes in pulmonary function studies which prompted discontinuation of the bleomycin.

DISCUSSION

This empiric clinical trial did not improve on the response rate in disseminated melanoma. With proliferation kinetic principles dictating this therapeutic protocol, the response rate of 21% is no different than that reported with numerous single agents, particularly DTIC. It is obvious that further exploration of these principles for the treatment of human malignancies is warranted and should continue.

REFERENCES

1. YOUNG RC, and DEVITA VT, JR. Cell cycle characteristics of human solid tumors in vivo. Cell Tissue Kinet 3:285, 1970.
2. HAGEMANN RF, and SCHIFFER LM. Cell kinetic analysis of a human melanoma in vitro and in vivo-vitro. JNCI 47:519, 1971.
3. BARRANCO SC, and HUMPHREY RM. The effects of bleomycin on survival and cell progression in Chinese hamster cells in vitro. Cancer Res 31:1218, 1971.
4. BARRANCO SC, LUCE JK, ROMSDAHL MD, ET AL. Bleomycin as a possible synchronizing agent for human cells in vivo. Cancer Res 33:882, 1973.

5. Costanzi J, Vaitkevicius V, Quagliana J, et al. Combination chemotherapy for disseminated malignant melanoma. Cancer 35:342, 1975.

John Costanzi[2,*]
Carol Fabian[3]
Henry Wilson[4]
Dennis Dixon[5]

Southwest Oncology Group

May 18, 1981

[2]University of Texas Medical Branch, Galveston.
[3]University of Kansas Medical Center, Kansas City.
[4]Ohio State University Hospital, Columbus.
[5]Southwest Oncology Group Statistical Office, Houston, TX.
Reprint requests to: Dr. John J. Costanzi (7608), c/o Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, KS 66103.

Phase II Study of Chlorozotocin in Non-Small Cell Carcinoma of the Lung[1]

Chlorozotocin, a 2-chloroethyl-substituted nitrosourea, has shown activity in animals against L1210 leukemia, P388 leukemia, B16 melanocarcinoma, and Lewis lung carcinoma (1,2). Phase I studies have shown mild marrow toxicity with both leukopenia and thrombocytopenia (3). Comparable doses of another nitrosourea, BCNU, showed a greater degree of marrow toxicity. Infrequent objective responses in human lung carcinoma have been reported with chlorozotocin (4,5). A phase II study of chlorozotocin was undertaken in the Cancer and Leukemia Group B to estimate its effectiveness in non-small cell lung carcinoma.

[1]This study was conducted by the Cancer and Leukemia Group B (*J. F. Holland*, chairman) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Dartmouth Medical School, Hanover, NH (*C. J. Cornell, Jr*, CA-04326); Georgetown University Hospital (*D. Hoth*); Cancer and Leukemia Group B Statistical Office (*T. F. Pajak*, CA-16118); Kantonsspital, Zurich, Switzerland (*G. Marz*); North Shore Hospital, Manhasset, NY (*D. R. Budman*, CA-07968); Rhode Island Hospital, Providence (*L. A. Leone*, CA-08025); Roswell Park Memorial Institute, Buffalo, NY (*E. S. Henderson*, CA-02599); Thomas Jefferson University Hospital, Philadelphia, PA (*F. Haurani*, CA-05462); State University of New York (SUNY)-Downstate, Brooklyn (*P. Bergevin*, CA-25119); Walter Reed Army Medical Center, Washington, DC (*J. Berenberg*); West Virginia University Medical Center, Morgantown (*P. C. Raich*, CA-28652); Long Island Jewish Medical Center, New Hyde Park, NY (*E. Cortes*, CA-11028); Columbia University-Presbyterian Medical Center, New York, NY (*R. R. Ellison*, CA-12011); University of Minnesota, Minneapolis (*N. J. Vogelzang*, CA-16450); University of California, San Diego (*M. Green*, CA-16450); University of Missouri, Columbia (*P. Henry*, CA-12046); and SUNY-Upstate Medical Center, Syracuse (*A. J. Gottlieb*, CA-21060).

MATERIALS AND METHODS

Patients with histologically proven non-small cell lung carcinoma and objectively measurable disease were considered eligible for the study. Only patients not considered suitable candidates for primary treatment with either surgery or radiotherapy were studied. In addition, patients were required to have demonstrated bone marrow recovery from prior therapy before entry in the study and to have a creatinine clearance of > 70 ml/minute. Informed consent was obtained.

Chlorozotocin was administered by rapid iv infusion at an initial dose of 120 mg/m^2 and was repeated every 6 weeks until disease progression or relapse from response occurred. Subsequent doses were adjusted according to the severity of toxicity.

Clinical, radiologic, and laboratory evaluations were performed at the beginning of the study and at 6-week intervals. *Marginal response* was defined as a 25%–49% reduction of all measurable lesions for at least 6 weeks; *stable disease* was defined as no measurable change in lesions with subjective and performance score stability or improvement; and *progressive disease* was defined as evidence of increase in lesions.

RESULTS

Among the 67 evaluable patients, 27 patients had adenocarcinoma, 28 had epidermoid carcinoma, and 12 had large cell carcinoma. Twenty-eight patients had had no prior chemotherapy, and 37 had initial performance scores of 0–1. Only 18 patients had both of these favorable characteristics. Eight of 39 patients with prior chemotherapy had exposure to the nitrosourea CCNU. Forty-five patients had had prior radiotherapy.

Therapeutic Effects

Three patients had marginal responses (one with adenocarcinoma, one with epidermoid carcinoma, and one with large cell carcinoma), and six had stable disease (two with adenocarcinoma, three with epidermoid carcinoma, and one with large cell carcinoma). Among the 18 patients with good initial performance scores and without prior chemotherapy, one had marginal response (6%) and three had stable disease (17%).

Toxicity

Nausea and vomiting were reported in 18% of the patients, with two patients requiring dose modifications. Frequency of severe leukopenia and throm-

TABLE 1.—Summary of observed toxic effects of chlorozotocin

Toxic effect	No. of evaluable patients	% of patients without toxic effect	% of patients with toxic effect			
			Mild	Moderate	Severe	Life-threatening
Leukopenia	51	88	12	0	0	0
Thrombocytopenia	51	68	14	16	2	0
Nausea and vomiting	45	82	11	5	2	0

bocytopenia associated with chlorozotocin were 0 and 2%, respectively, with nine patients requiring some dose modifications (table 1). In fact, only nine (18%) of 51 evaluable patients for toxicity showed moderate and severe leukopenia or thrombocytopenia.

DISCUSSION

Of the 67 chlorozotocin patients evaluable for response, there were three (4%) marginal responders and six (9%) with stable disease. This lack of therapeutic benefit, especially among the 18 chlorozotocin patients with the most favorable characteristics, indicates that the true partial and complete response frequency with the drug schedule tested is < 15% in non-small cell carcinoma of the lung ($P = 0.054$). Other studies have reported similar results in a small number of patients (4,5).

Since only 18% of the patients developed moderate to severe leukopenia or thrombocytopenia, the tested chlorotozocin schedule could have started with a higher initial dose. Whether chlorozotocin will be active in a different schedule, at higher doses, or in combination with other agents needs to be determined.

REFERENCES

1. CARTER SK. An overview of the status of the nitrosoureas in other tumors. Cancer Chemother Rep Part 3, vol 4 (4):35–46, 1973.
2. MOERTEL CG. Therapy of advanced gastrointestinal cancer with the nitrosoureas. Cancer Chemother Rep Part 3, vol 4 (4):27–34, 1973.
3. HOTH D, WOOLLEY P, GREEN D, ET AL. Phase I studies on chlorozotocin. Clin Pharmacol Ther 23:712–722, 1978.
4. CREAGAN ET, EAGAN RT, FLEMING TR, ET AL. Phase II evaluation of chlorozotocin in advanced bronchogenic carcinoma. Cancer Treat Rep 63:2105–2106, 1979.
5. CASPER ES, and GRALLA RJ. Phase II evaluation of chlorozotocin in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 63:549–550, 1979.

Cornelius J. Cornell, Jr *
Daniel Hoth
Thomas F. Pajak

Cancer and Leukemia Group B

May 20, 1981

*Reprint requests to: Cornelius J. Cornell, Jr, MD, Section of Hematology/Oncology, Department of Medicine, Dartmouth Medical School, Hanover, NH 03755.

Phase II Trial of 5-FU Plus Melphalan in Advanced Colorectal Carcinoma[1]

Twenty-five patients with advanced colorectal carcinoma were given 5-FU (10 mg/kg) weekly by iv bolus plus melphalan (0.1 mg/kg/day) orally for 5 consecutive days beginning after the first 5-FU dose and repeated every 4 weeks. Details of patient selection, monitoring, and dose modification have been reported (1). Patients (table 1) received treatment for 5–64 weeks and 17 patients received at least 12 weeks of treatment. Toxic effects were as follows: wbc count < 4000/mm^3 (ten patients), platelet count < 100,000/mm^3 (four), nausea and/or vomiting (seven), and stomatitis (two). Treatment was unusually well-tolerated and in no case was interrupted because of toxicity.

The objective response rate was 24%, comprising one complete response (duration, 56 weeks) and five partial responses (mean response duration, 18 weeks; range, 4–36). Mean survival times were as follows: objective remission (six patients)—54 weeks (range, 38–93); stable disease (eight patients)—30 weeks (range, 20–54); and disease progression (11 patients)—19 weeks (range, 6–37). Objective responders survived longer than nonresponders (Mann-Whitney U test, $P < 0.001$).

Two previous studies (2,3) concluded that the combination of 5-FU and melphalan was not useful

[1]Supported by the University of Queensland Cancer Research Fund.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	25
Females	13
Males	12
Age (yrs)	
Mean: 56.9	
Range: 34–76	
Primary site	
Colon	15
Rectum	10
Metastatic sites	
Local + peritoneal	12
Liver	17
Other visceral	10
Multiple sites	13

in the management of colorectal carcinoma. In the study of Berman et al (2) the melphalan was given before the 5-FU, the toxicity was marked, and no objective regression was noted in 14 patients. However, these drugs have been shown experimentally (4) to have a synergistic action against L1210 murine leukemia which was most marked when melphalan was sequenced after 5-FU as in the clinical study of Presant et al (3) in which the response rate was 18%.

It has been demonstrated that iv administration of melphalan is preferable to oral administration (5). We consider that further evaluation of this combination for colorectal carcinoma is warranted, with iv melphalan sequenced after 5-FU.

REFERENCES

1. GOUGH IR, FURNIVAL CM, and BURNETT W. Melphalan and 5-fluorouracil in advanced gastrointestinal carcinoma: a preliminary report. Cancer Treat Rev 6 (suppl):127–129, 1979.
2. BERMAN R, GILES GR, MALHOTRA A, ET AL. Randomized trial of melphalan plus 5-fluorouracil (5-FU) versus methyl-CCNU plus 5-FU in patients with advanced colorectal cancer. Cancer Treat Rep 62:457–459, 1978.
3. PRESANT G, RATKIN G, and KLAHR C. Phase II trial of 5-fluorouracil plus melphalan in colorectal carcinoma. Cancer Treat Rep 62:461–462, 1978.
4. VALERIOTE E, VIETTI T, and COULTER D. Synergistic effect of 5-FU and L-PAM on L1210 leukemia. *In* Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells (Drewinko B, and Humphrey RM, eds). Baltimore, MD, Williams and Wilkins Co, 1977, pp 733–742.
5. TATTERSALL MHN, JARMAN M, NEWLANDS ES, ET AL. Pharmacokinetics of melphalan following oral or intravenous administration in patients with malignant disease. Eur J Cancer 14:507–513, 1978.

I. R. Gough
C. M. Furnival
G. S. J. Thynne
W. Burnett

Department of Surgery
University of Queensland
Royal Brisbane Hospital
Brisbane, Australia 4029

June 1, 1981

Oral Complications of Cancer Chemotherapy

Baltimore, Maryland, September 21–22, 1981

This two-day course will be held at the International Hotel, Baltimore-Washington International Airport, Baltimore, Maryland. The program will focus on a critical facet of supportive care of cancer patients, oral complications of cancer chemotherapy. Patients receiving chemotherapy often develop oral pathology that can be difficult to accurately diagnose and manage. The first day of the program will be devoted to aspects related to the supportive care of these patients. Topics to be covered include chemotherapy-induced oral complications, alterations in microbiologic flora, and principles of infection diagnosis and management. The second-day sessions will address the logistic, administrative, and financial aspects of establishing and maintaining a program in dental oncology. The course coordinators are Dr. Douglas E. Peterson and Dr. Stephen T. Sonis.

This course meets the criteria for 9 credit hours in Category I of the Physician's Recognition Award of the AMA and the ACCME provided it is used and completed as designed. This program is acceptable for 9 hours of maintenance, fellowship, or mastership credit in the Academy of General Dentistry.

For further information contact: Program of Continuing Education, 10 S Pine St, MSTF 300, Baltimore, MD 21201 (phone: 301–528-3956).

Eighth Annual Conference on Medical Oncology

Perugia, Italy, October 6–10, 1981

This conference, sponsored by the Italian Association of Medical Oncology, will be held October 6–10, 1981, at the University of Perugia. *Direct inquiries to:* Prof. Maurizio Tonato, Division of Medical Oncology, Ospedale Generale Regionale, 06100 Perugia, Italy (075/608456).

San Antonio Fourth Annual Breast Cancer Symposium

San Antonio, Texas, November 6–7, 1981

This symposium will be held at La Mansion del Norte, San Antonio, Texas. Symposium coordinators are William L. McGuire, MD, and Charles A. Coltman, Jr, MD. AMA credit has been approved. *For more information contact:* Marilyn Rennels, Office of Continuing Education, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78284 (512–691-6295).

Current Concepts in Cancer Management: Successful Treatment and Its Consequences

San Francisco, California, November 13–14, 1981

Current Concepts in Cancer Management: Successful Treatment and Its Consequences, a postgraduate symposium sponsored by the Claire Zellerbach Saroni Tumor Institute of Mount Zion Hospital and Medical Center, will be held November 13–14, 1981, at the Fairmont Hotel, San Francisco, California.

The course has been approved for up to 11 hours of Category I credit under the California Medical Association's Program of Continuing Education. Tuition is $200 ($150 before September 15, 1981), and advance registration is required.

Guest faculty will include Drs. Stephen K. Carter, Vincent T. DeVita, Lawrence H. Einhorn, Ruth H. Grobstein, Henry S. Kaplan, Donald L. Morton, Jean Papillon, Saul A. Rosenberg, and Jordan R. Wilbur.

For further information, contact: Claire Zellerbach Saroni Tumor Institute of Mount Zion Hospital and Medical Center, P.O. Box 7921, San Francisco, CA 94120 (415–567-6600, ext. 2125).

Revisions for the Directory of Training Programs in Internal Medicine: Residency and Subspecialty Fellowships

Internal medicine subspecialty fellowship training programs in the United States are listed in "Directory of Training Programs in Internal Medicine: Residency and Subspecialty Fellowships" (Washington, DC, Association of Professors of Medicine, 1979). The Directory has been distributed to all directors of approved residency training programs in internal medicine and to all directors of subspecialty fellowship training programs. It has been used by residents and others who want information on the existence and location of the various programs.

To update the listing for 1981–1982, the National Study of Internal Medicine Manpower, in collaboration with the Association of Professors of Medicine, is calling upon directors of subspecialty fellowship training programs. The following need to be identified: the beginning of a subspecialty fellowship training program, the amalgamation of a program with another institution, or the phasing out of a program. If any of these changes has occurred in your training program, please submit the form below by October 15, 1981.

For the first time, Clinical Pharmacology, Geriatrics, and Nutrition fellowship training programs that admit internal medicine physicians will be included in this Directory. Therefore, directors of all such programs should return this form.

In order to be listed in the Directory, the program must have at least one full-time Fellow in 1981–1982 whose overall direction is provided by the program director listed below.

Please return the requested information to Alvin R. Tarlov, MD, Director, National Study of Internal Medicine Manpower, University of Chicago, 950 E 59th St, Box 265, Chicago, IL 60637.

NAME OF PROGRAM DIRECTOR _____

SUBSPECIALTY _____

HOSPITAL _____

ADDRESS _____

_____

PHONE NUMBER _____

Check One: This program is:

____ (1) a new program

____ (2) amalgamating with another (ANSWER A)

____ (3) phasing out or terminating the training of fellows.

(A) If Amalgamating:

NAME OF PROGRAM DIRECTOR _____

SUBSPECIALTY _____

NAME AND ADDRESS OF BASE HOSPITAL _____

☆ U.S. GOVERNMENT PRINTING OFFICE: 1981 — 341-140/102

a publication
of the
national cancer institute

NIH Publication No. 81-22